

Figure 1: Examples of Nuclease Stable Ribozyme Motifs

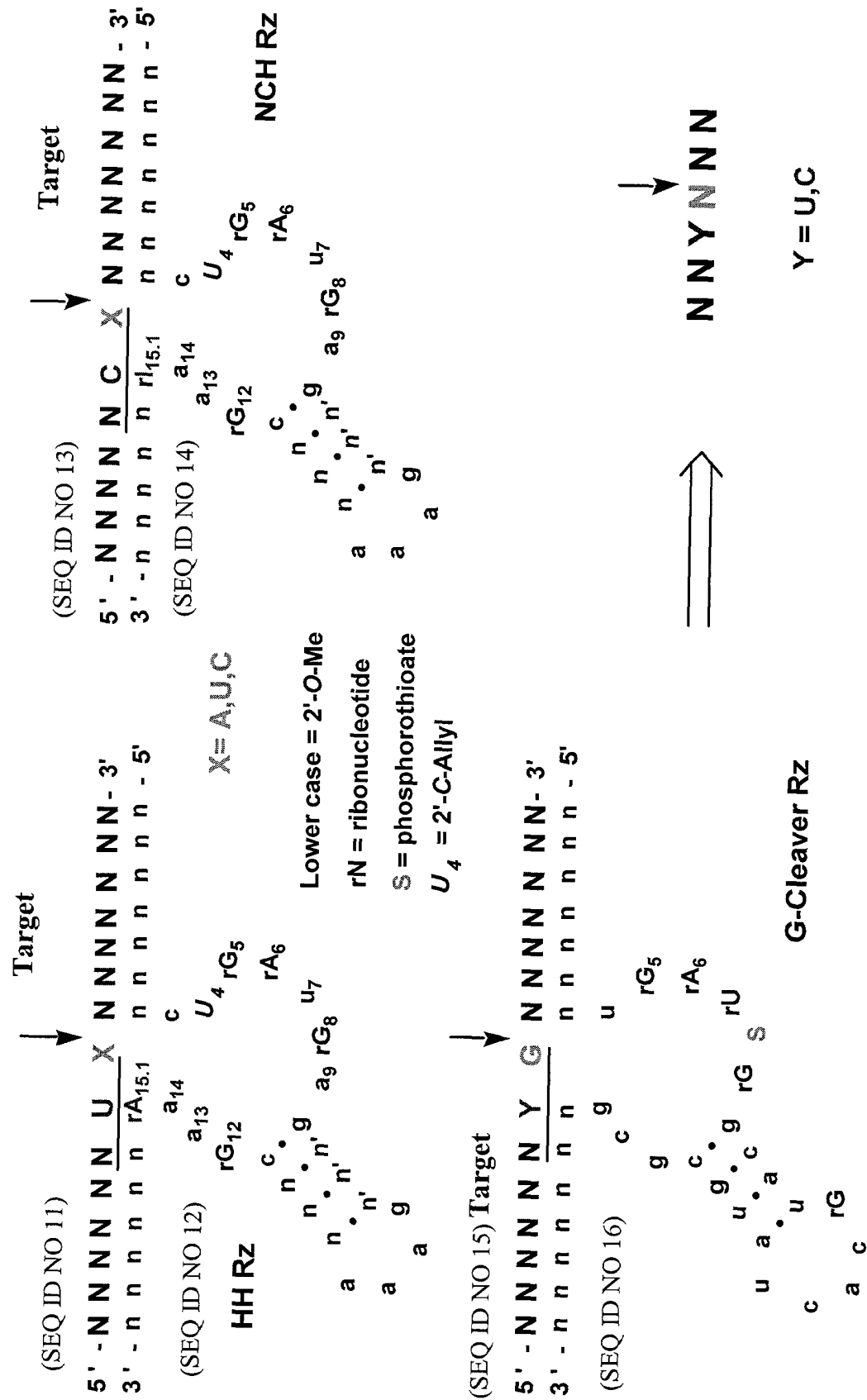


Figure 2: 2'-O-Me substituted Amberzyme Enzymatic Nucleic Acid Motif

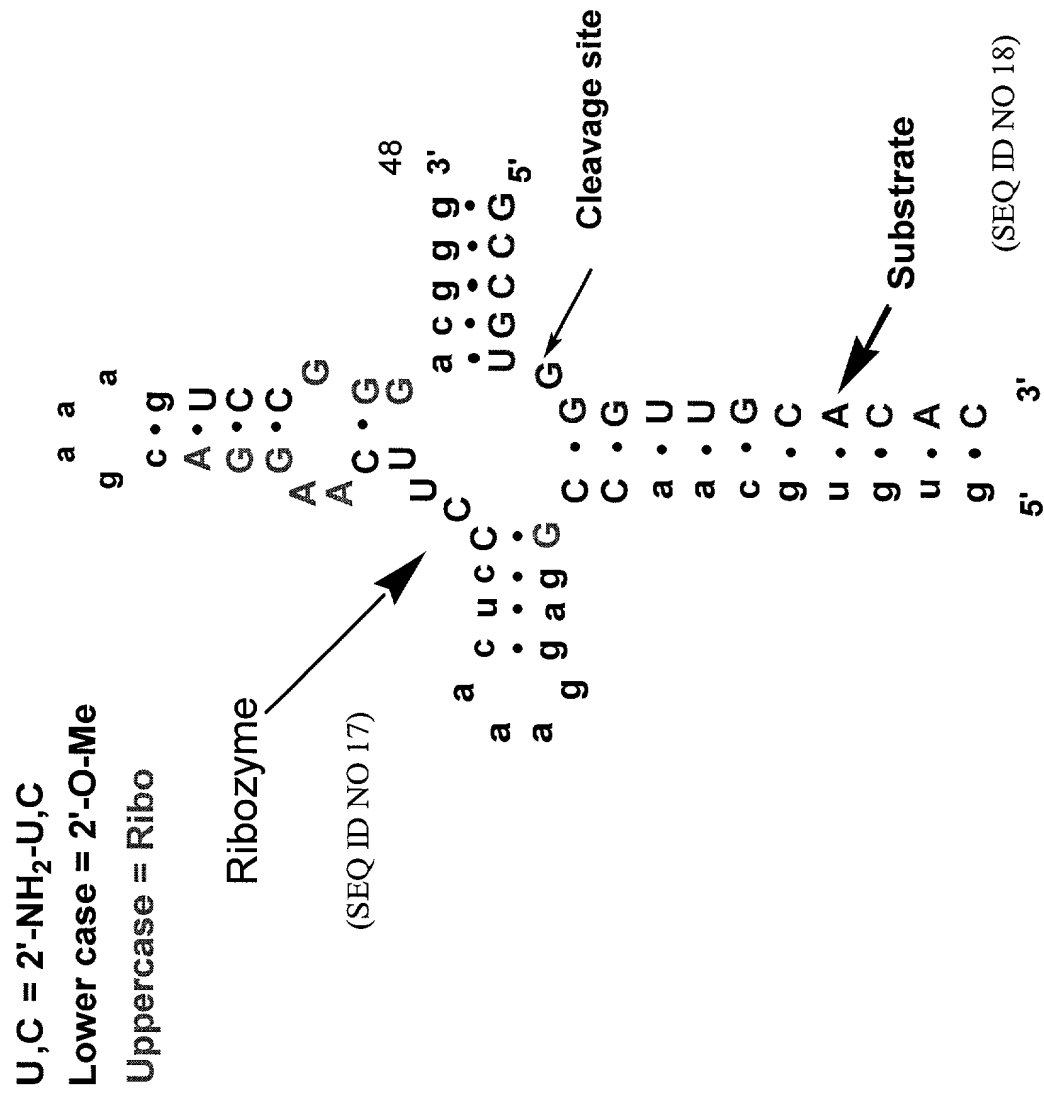
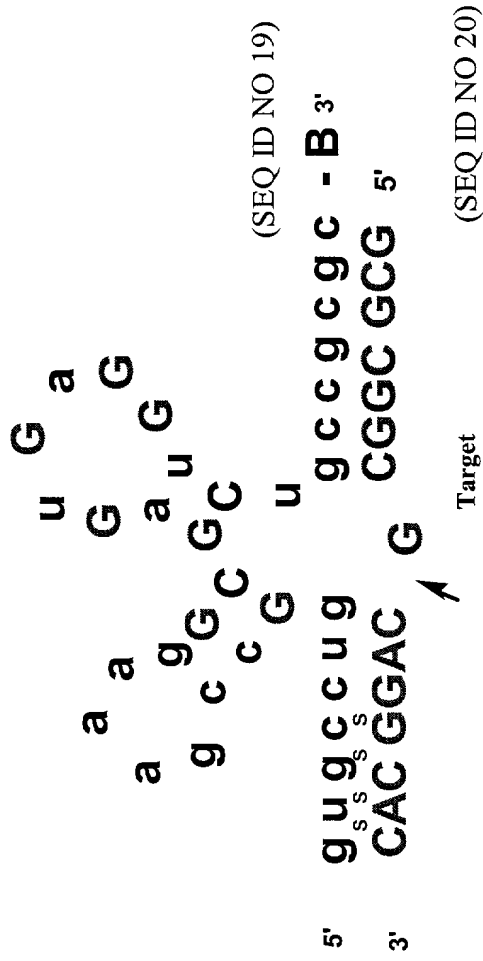


Figure 3: Stabilized Zinzyme Ribozyme Motif

## Zinzyme A-motif RZ



### Legend

Uppercase indicates natural ribo residues

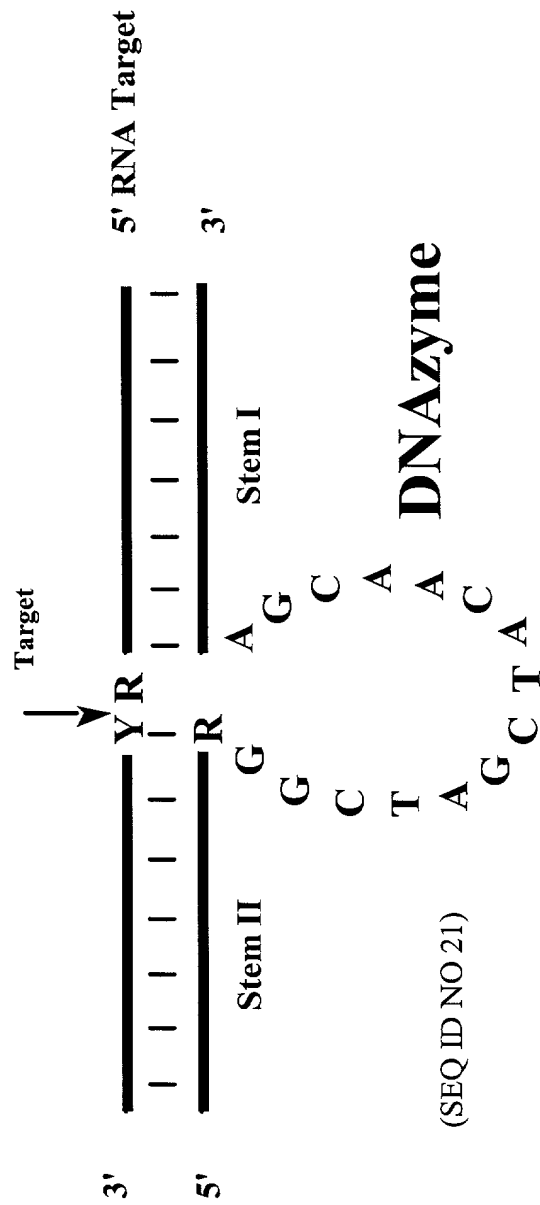
**C** indicates 2' - d-NH<sub>2</sub>-C

Lowercase: 2'-O- Me

Subscript <sub>s</sub> indicates phosphothioate linkage

B: 3'-3' abasic moiety

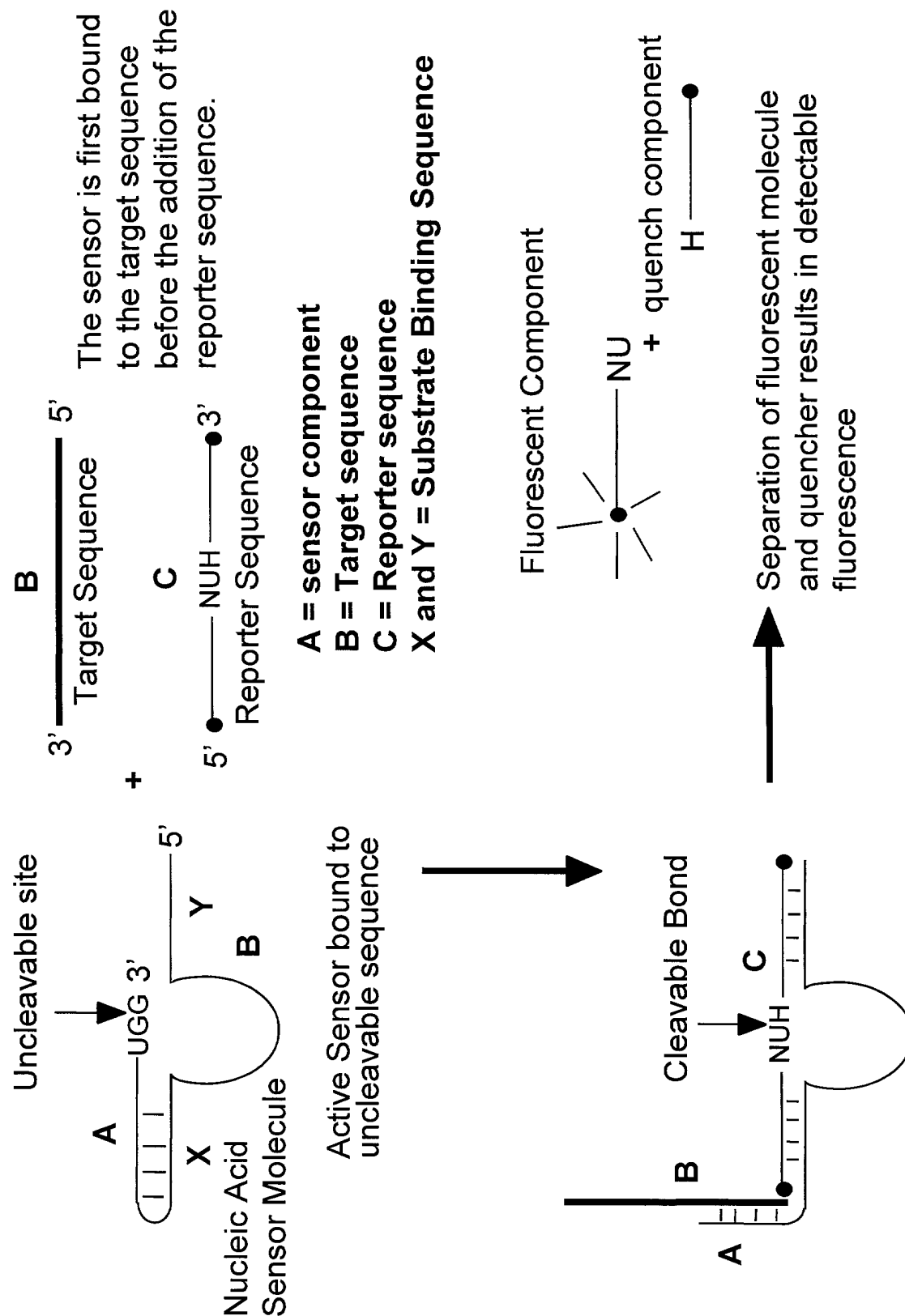
Figure 4: DNAzyme Motif



Legend

- Y = U or C
- R = A or G

**Figure 5. Detection of Target Sequence Using a Cis-Blocking Sequence**



**A.** Bound to the target sequence

Accessible by the reporter oligonucleotide

The enzymatic nucleic acid is unbound from its sensor component allowing for normal catalytic activity.

**B.**

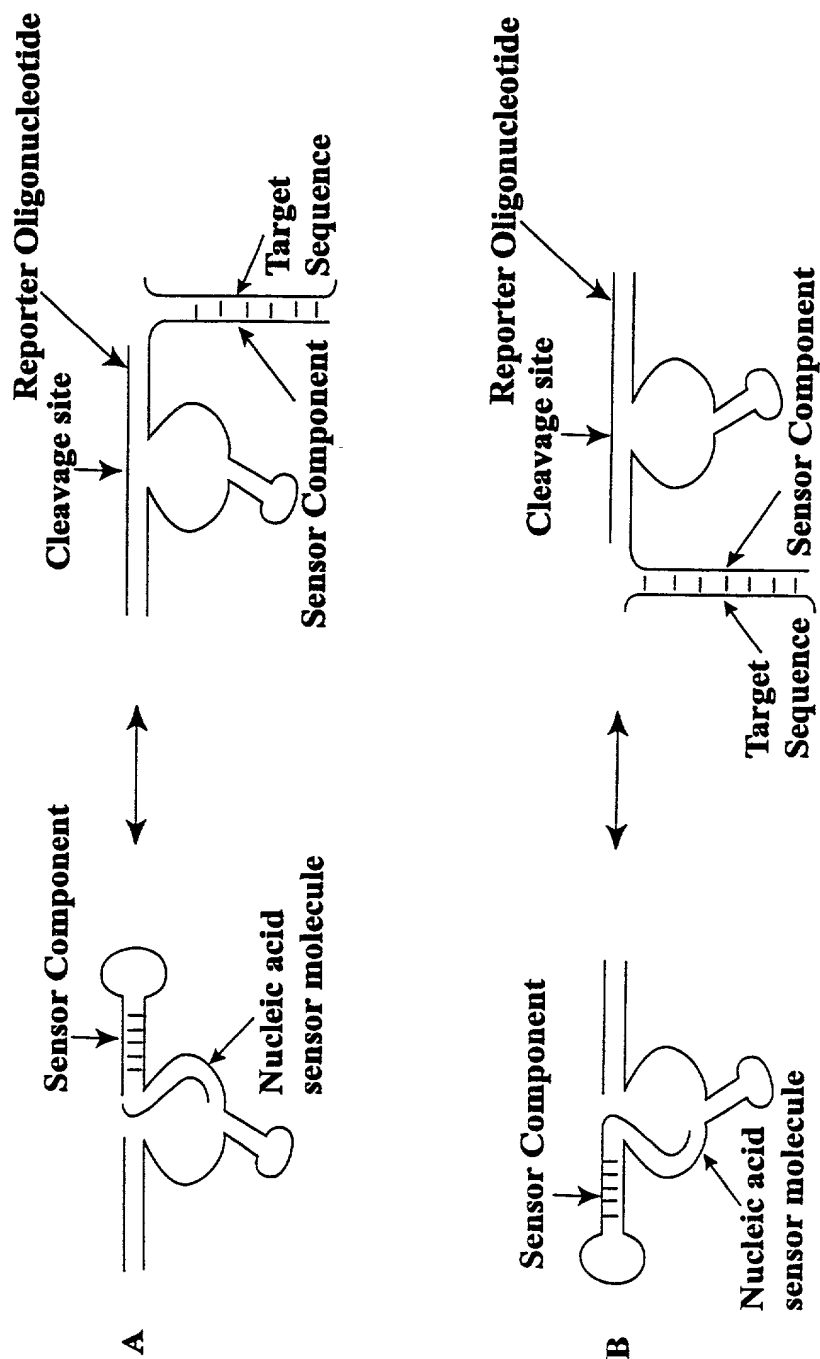
The nucleic acid catalyst binds to the sensor component which prevents the catalytic activity of the molecule. Binding is to either the substrate binding arms or to nucleotides within the sensor molecule core.

The catalytic region is now inaccessible to the reporter sequence

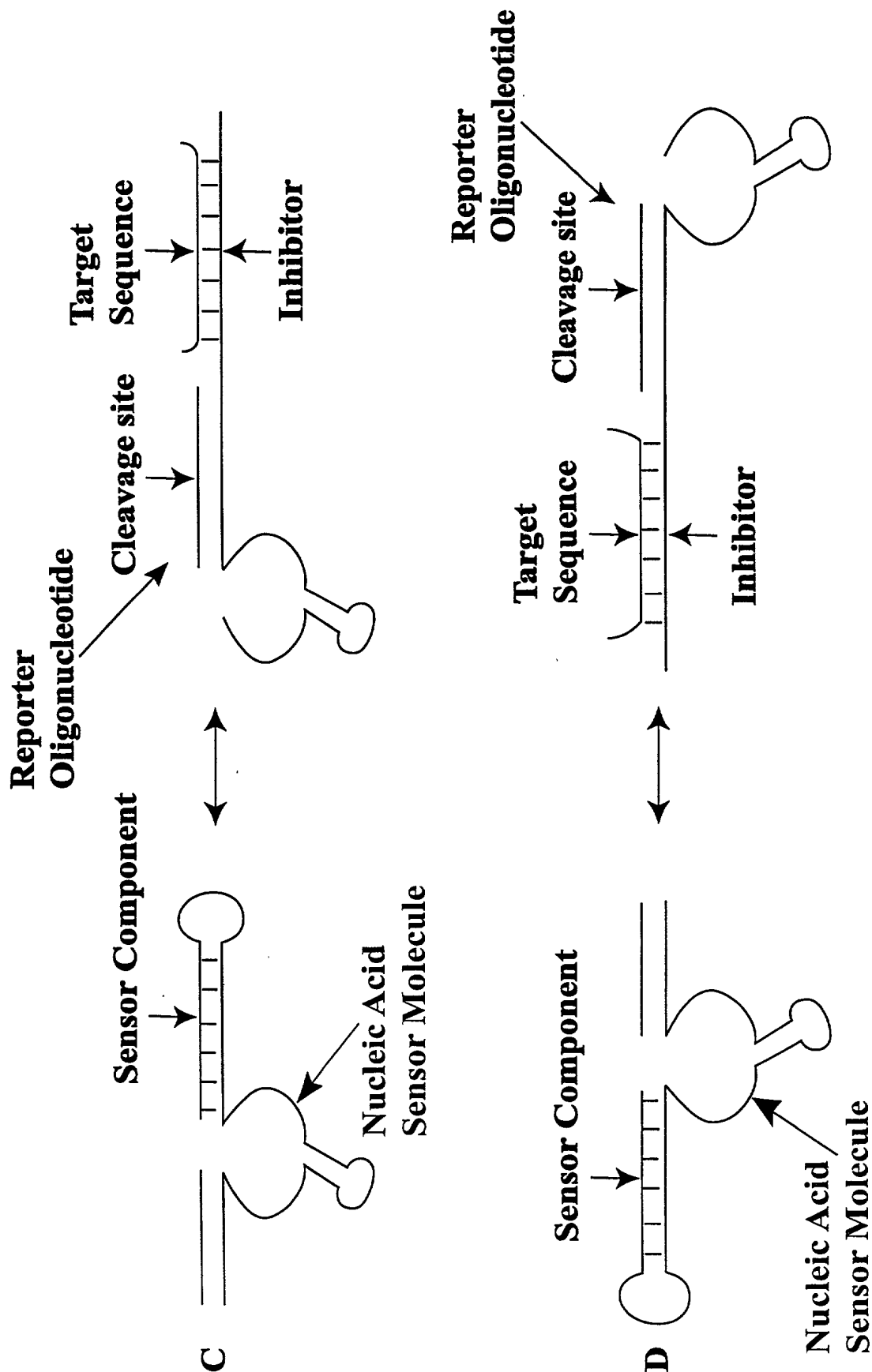
**Legend:**

- Sensor Component
- Nucleic Acid Catalyst
- Linker (Present or Absent)

**Figure 7a. Examples of Diagnostic Effector Molecules**

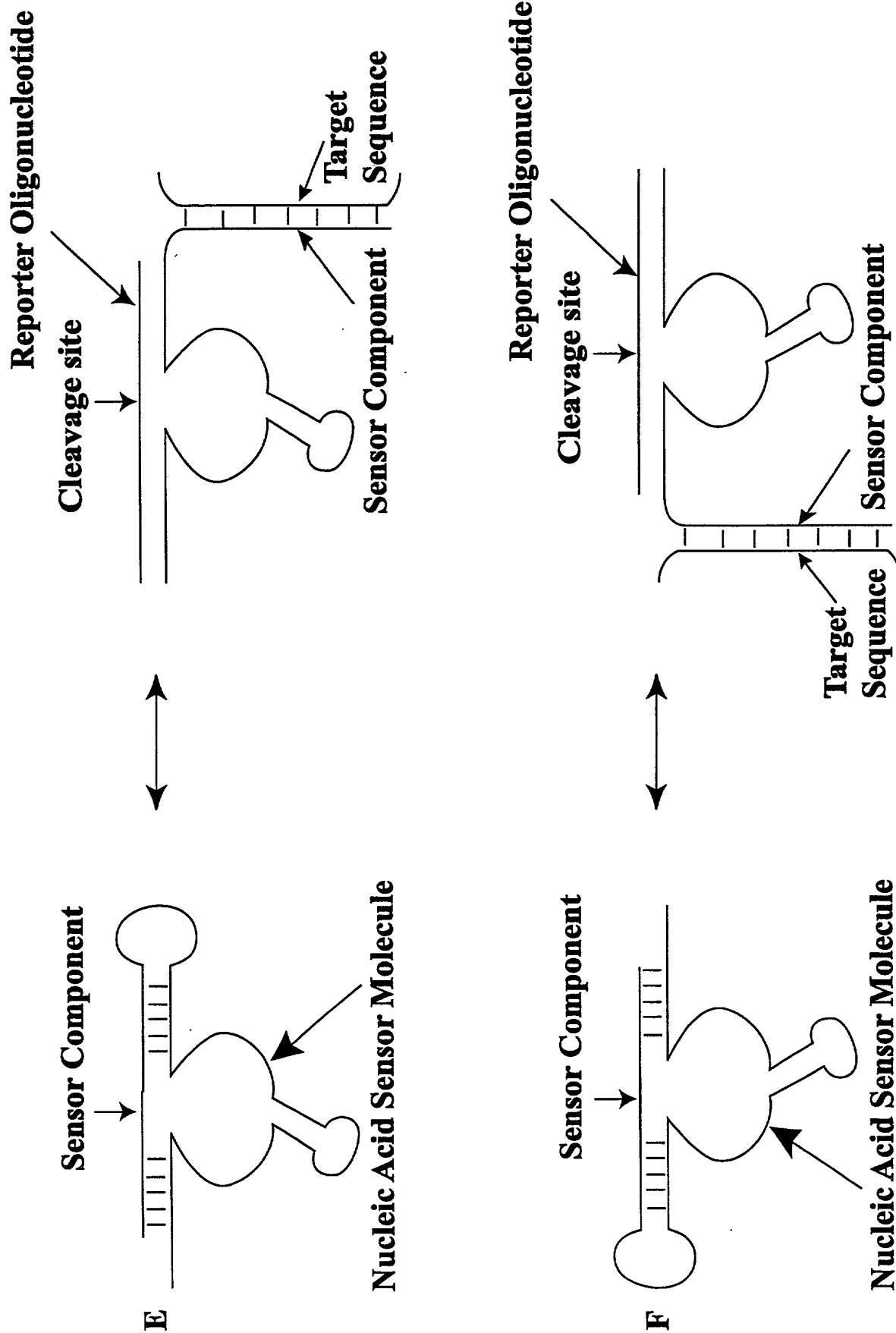


**Figure 7b. Examples of Diagnostic Effector Molecules**

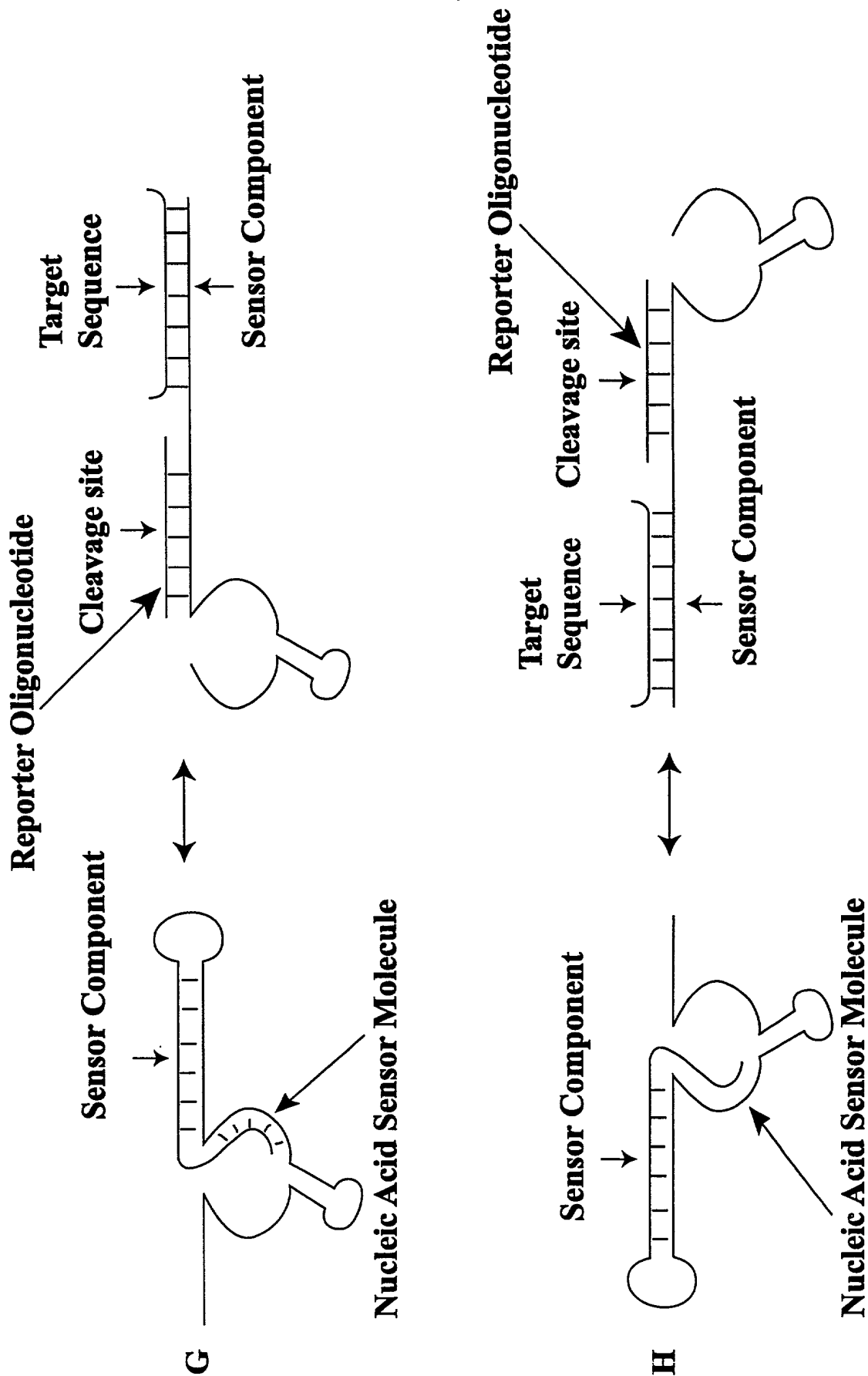




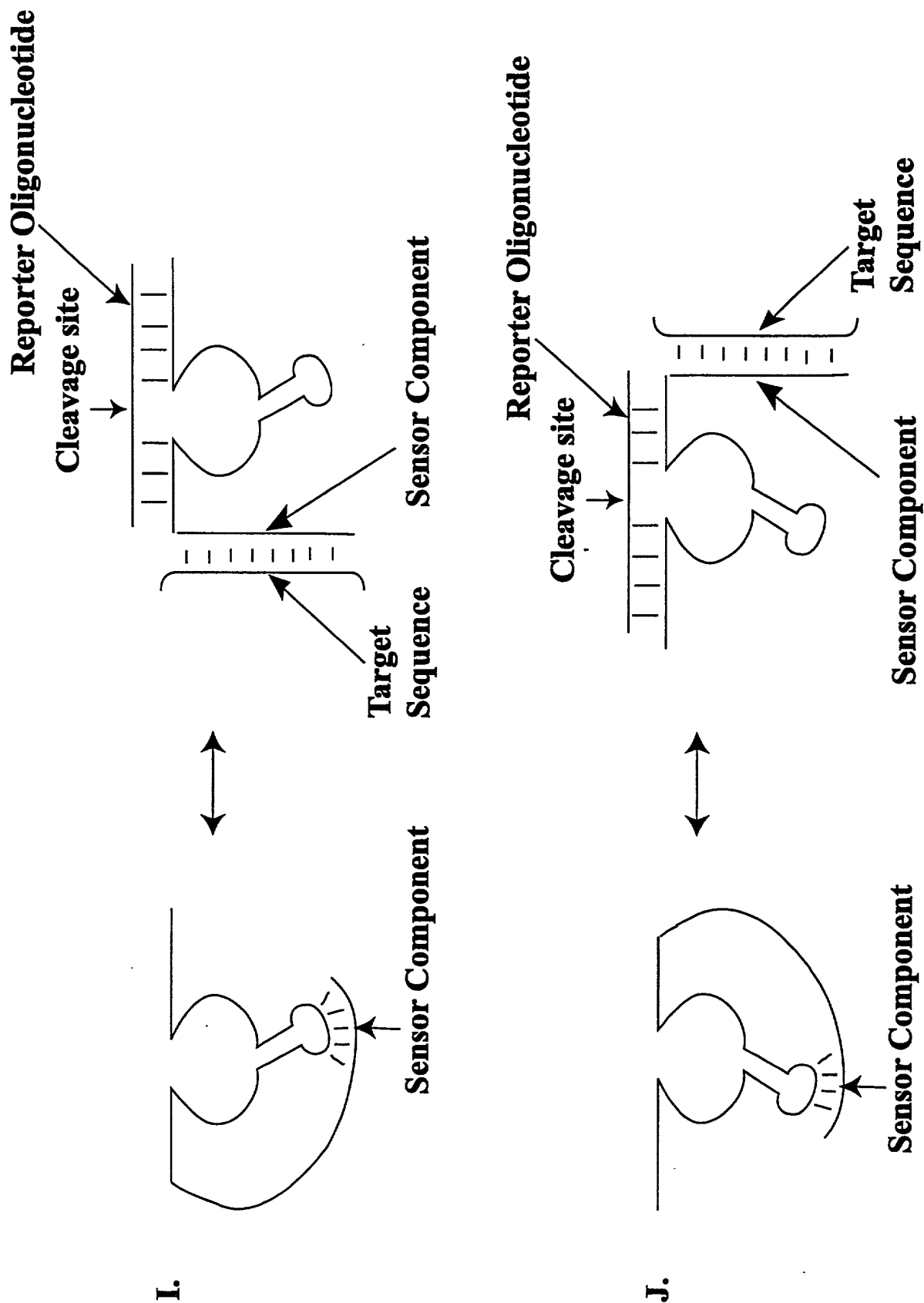
**Figure 8a. Examples of Diagnostic Effector Molecules**



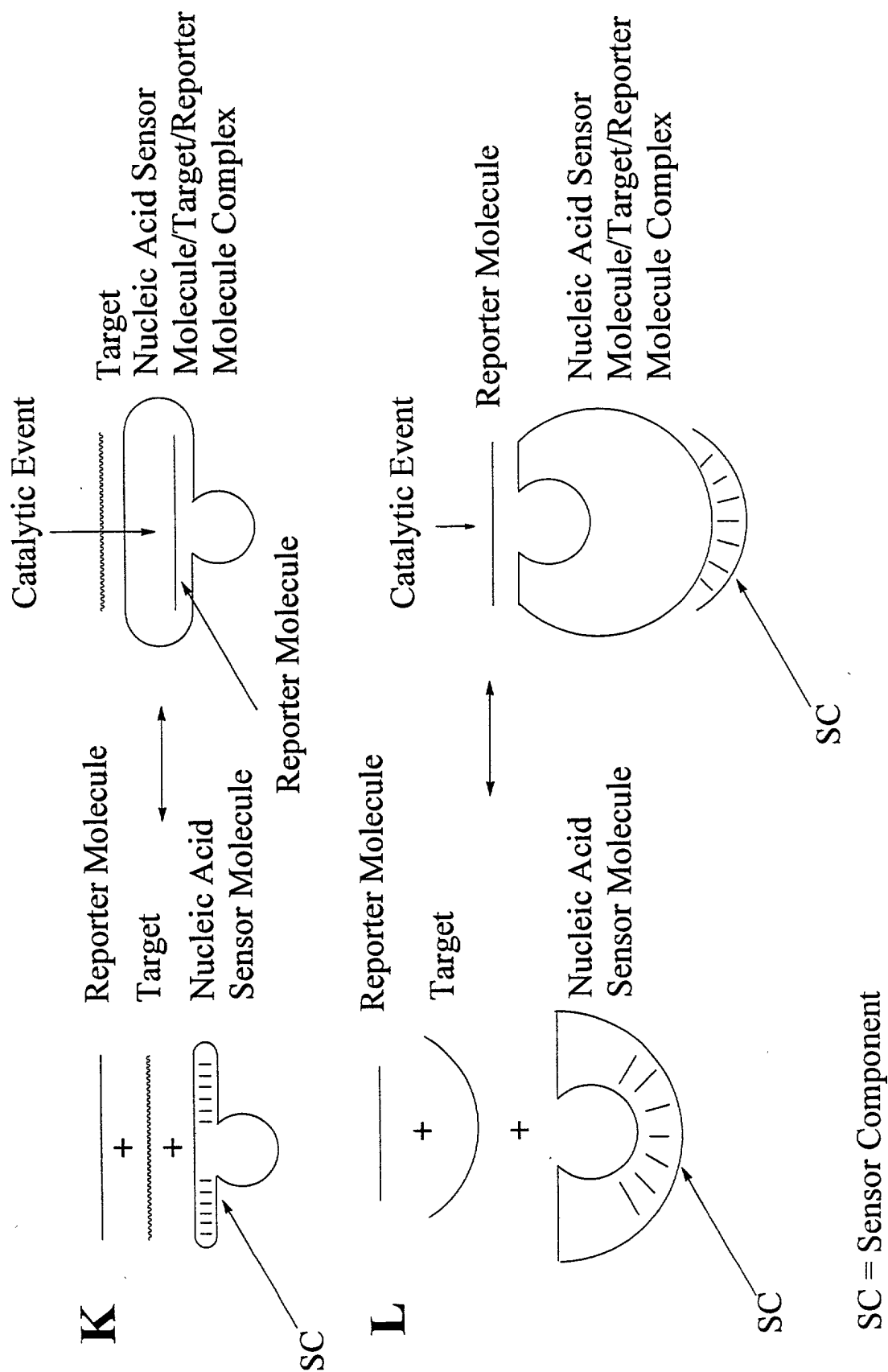
**Figure 8b. Examples of Diagnostic Effector Molecules**



**Figure 9. Examples of Diagnostic Effector Molecules**

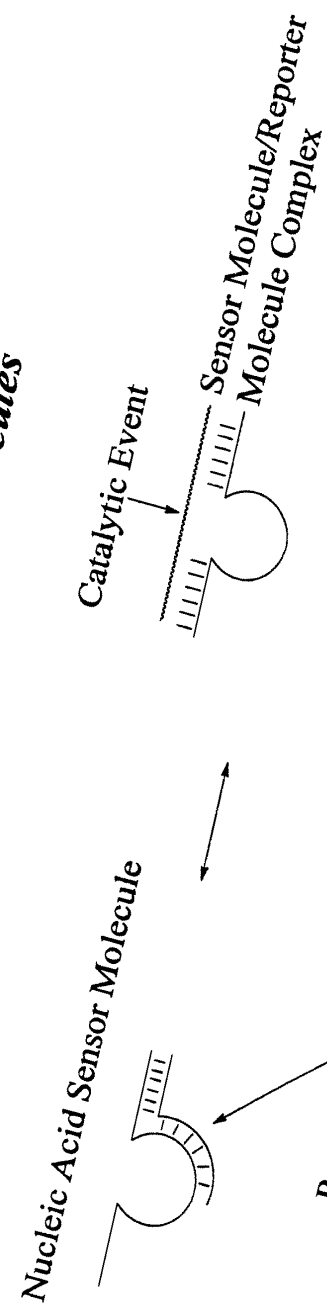


**Figure 10: Examples of Diagnostic Effector Molecules**

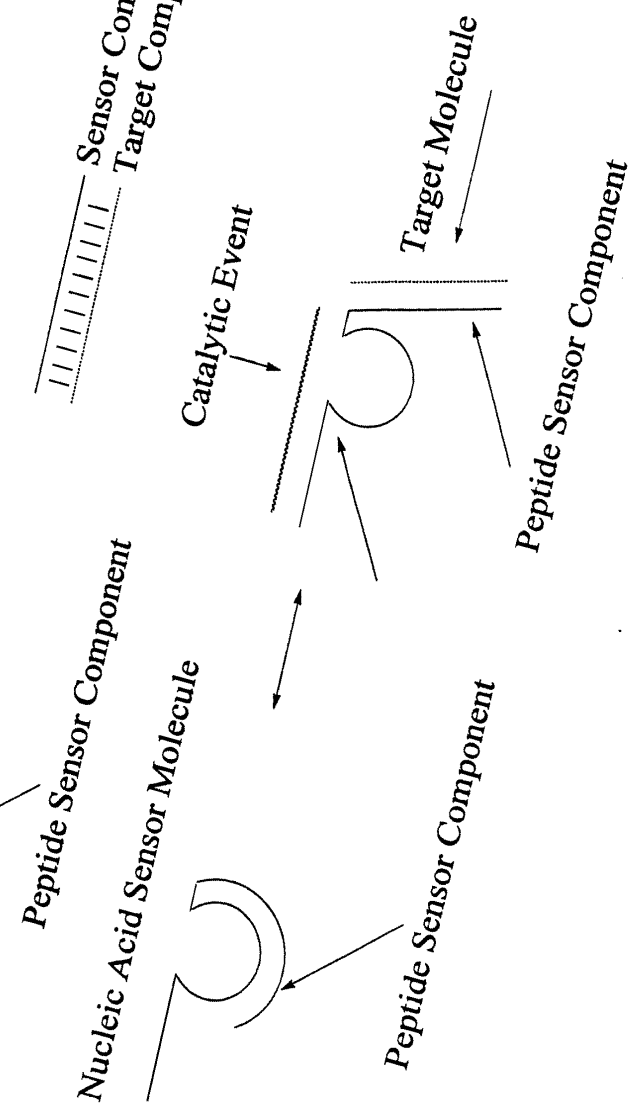


**Figure 11: Examples of Diagnostic Effector Molecules**

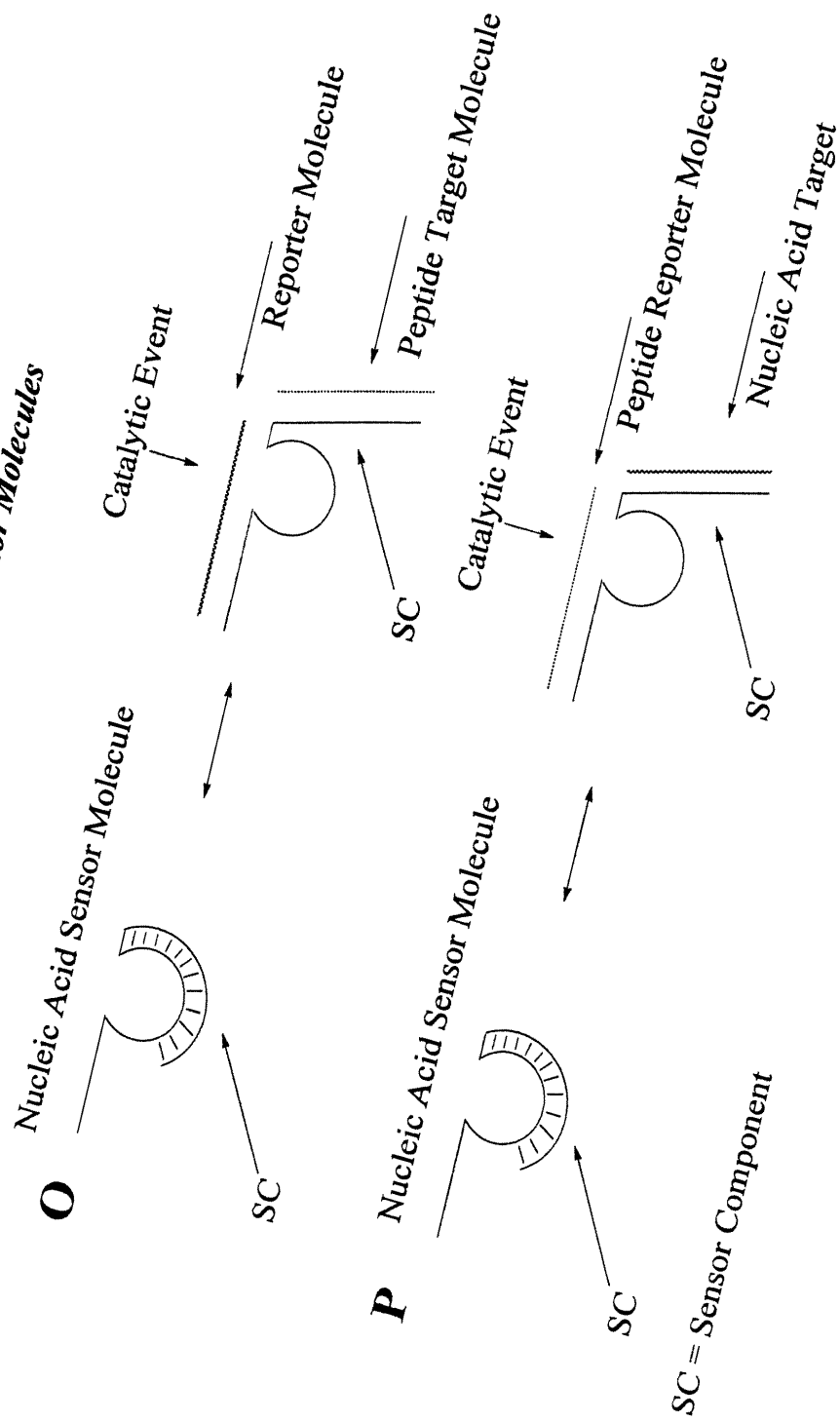
**M**



**N**

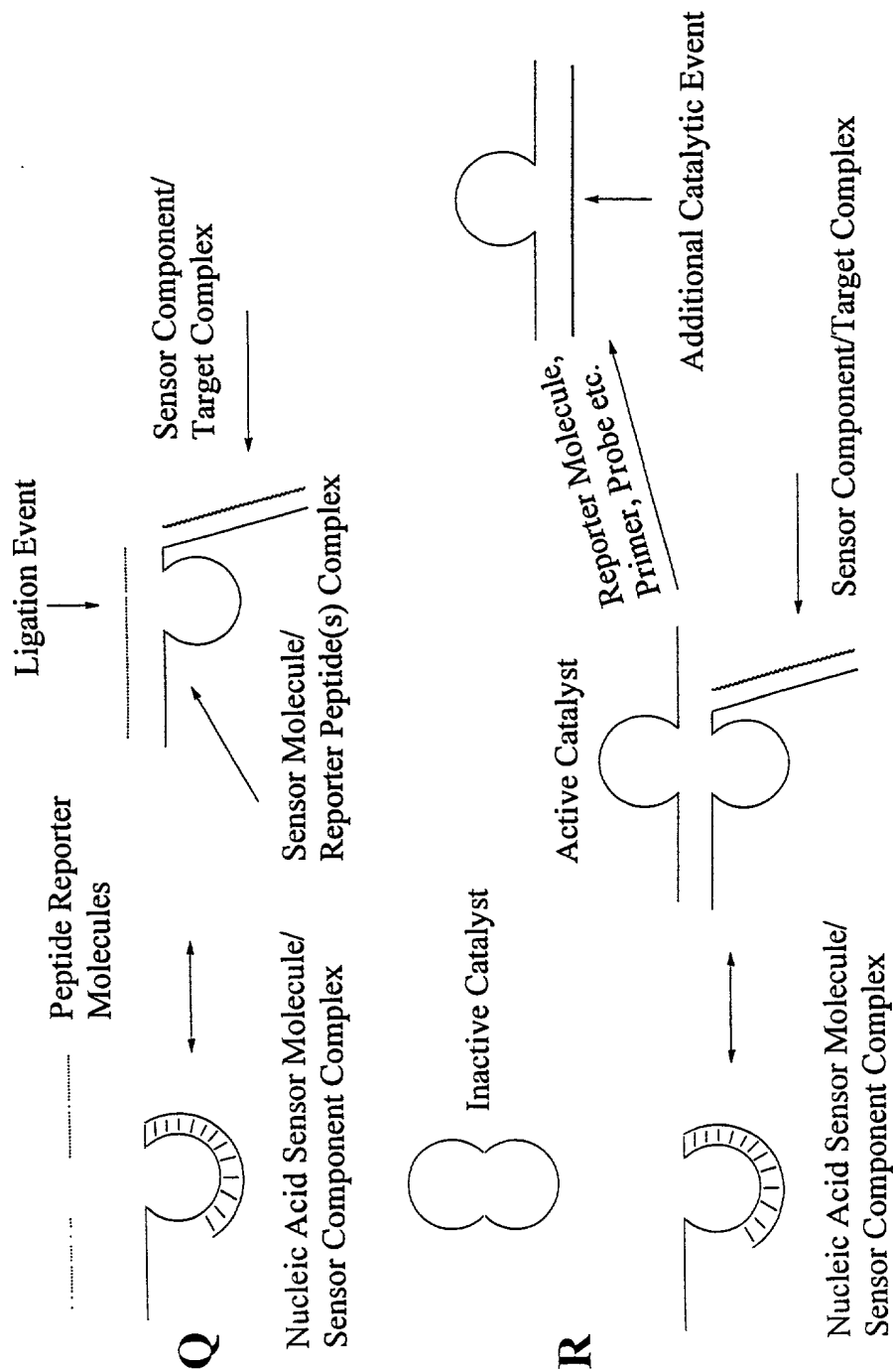


**Figure 12: Examples of Diagnostic Effector Molecules**

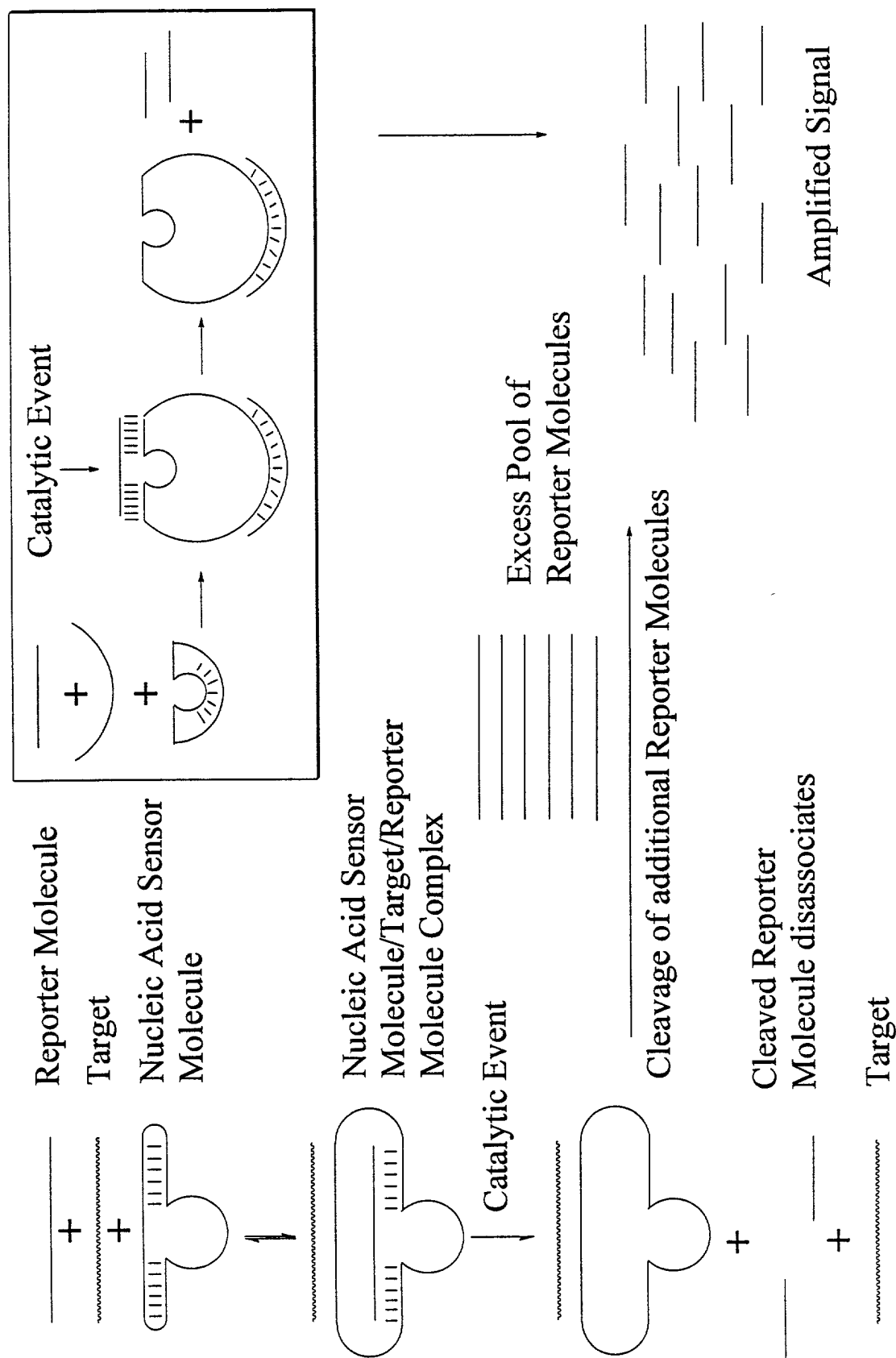


FOOT" 09T2550

**Figure 13: Examples of Diagnostic Effector Molecules**



**Figure 14: Inherent Amplification of Signal**





**Figure 15: Example of Diagnostic System**

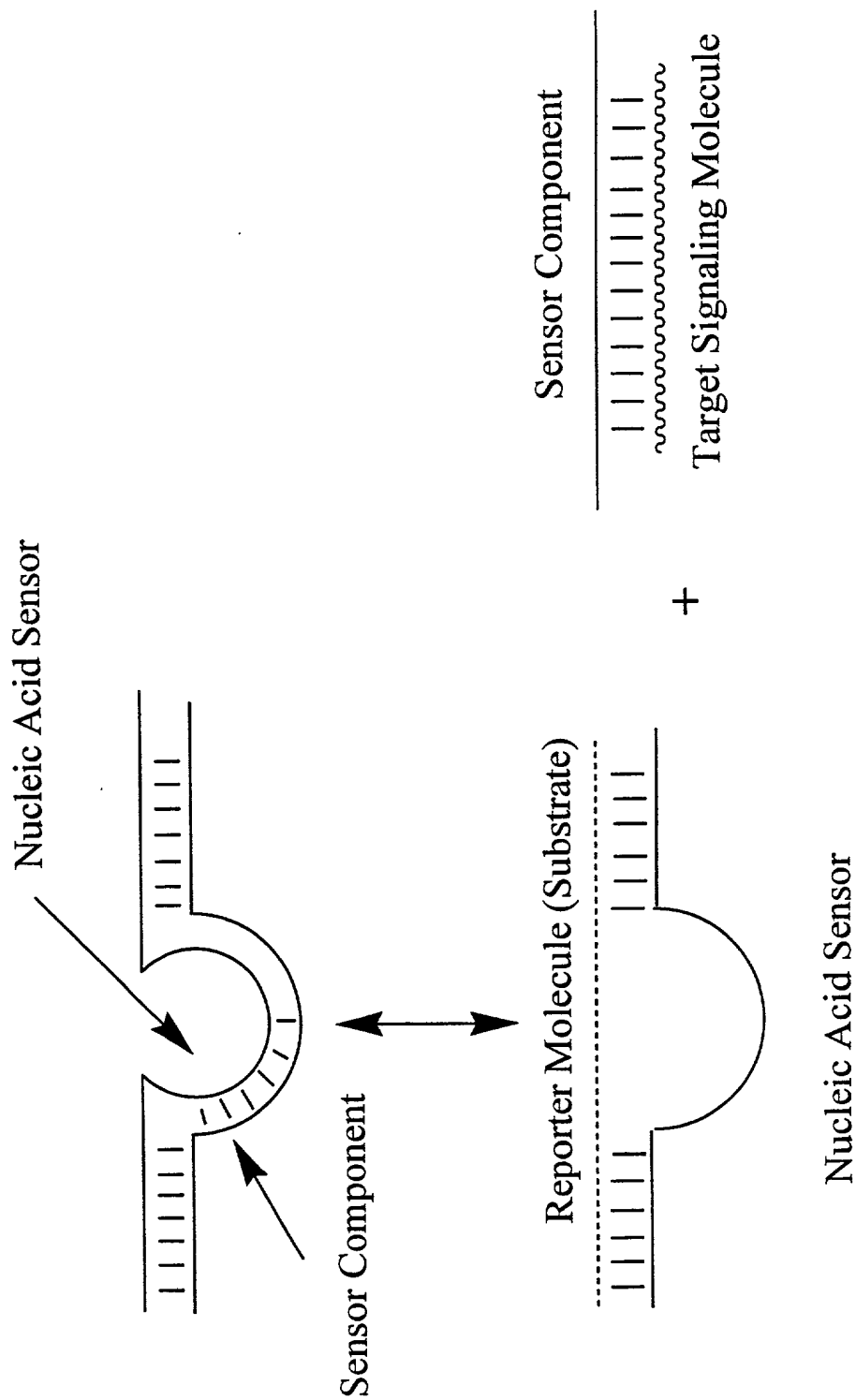
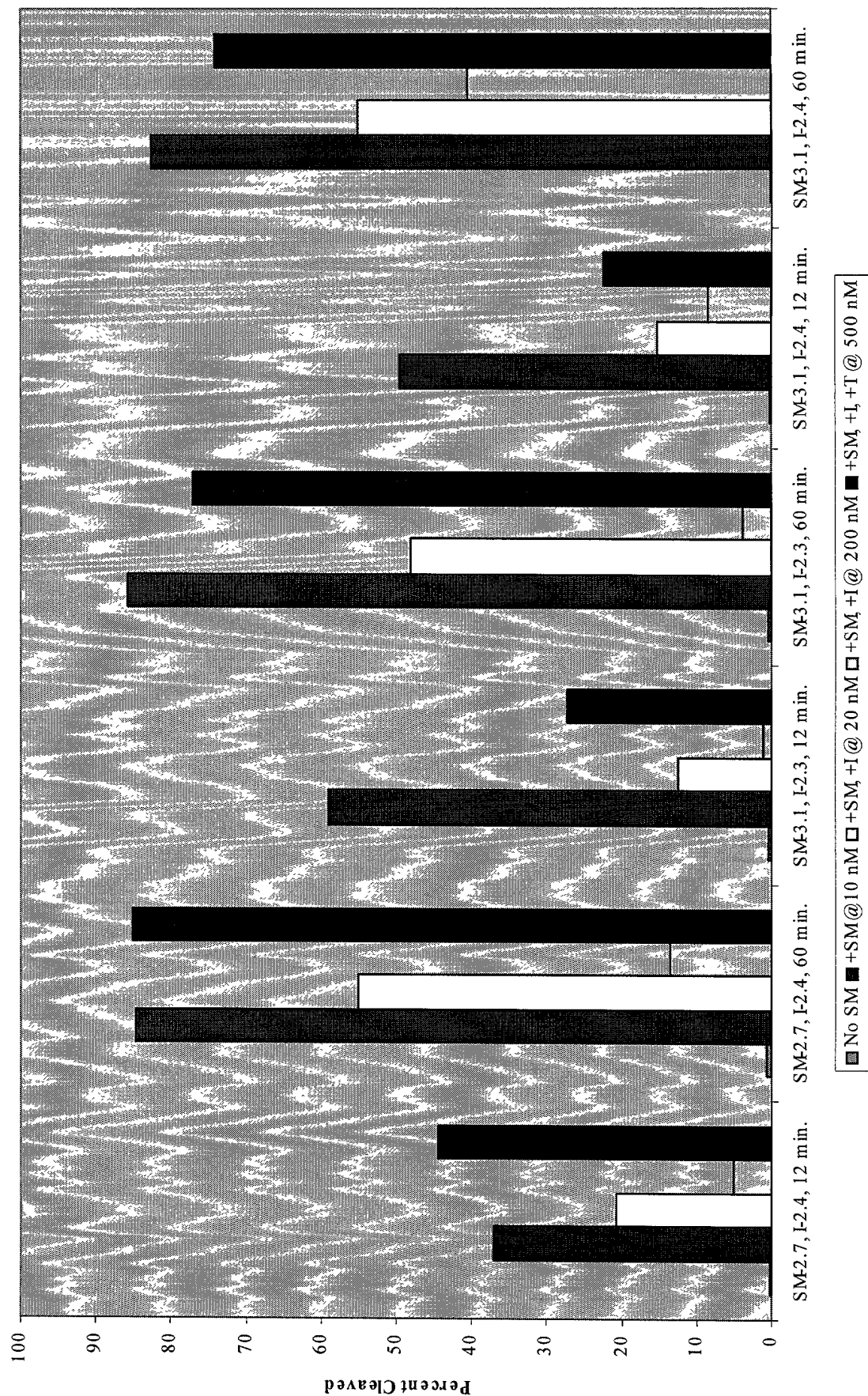
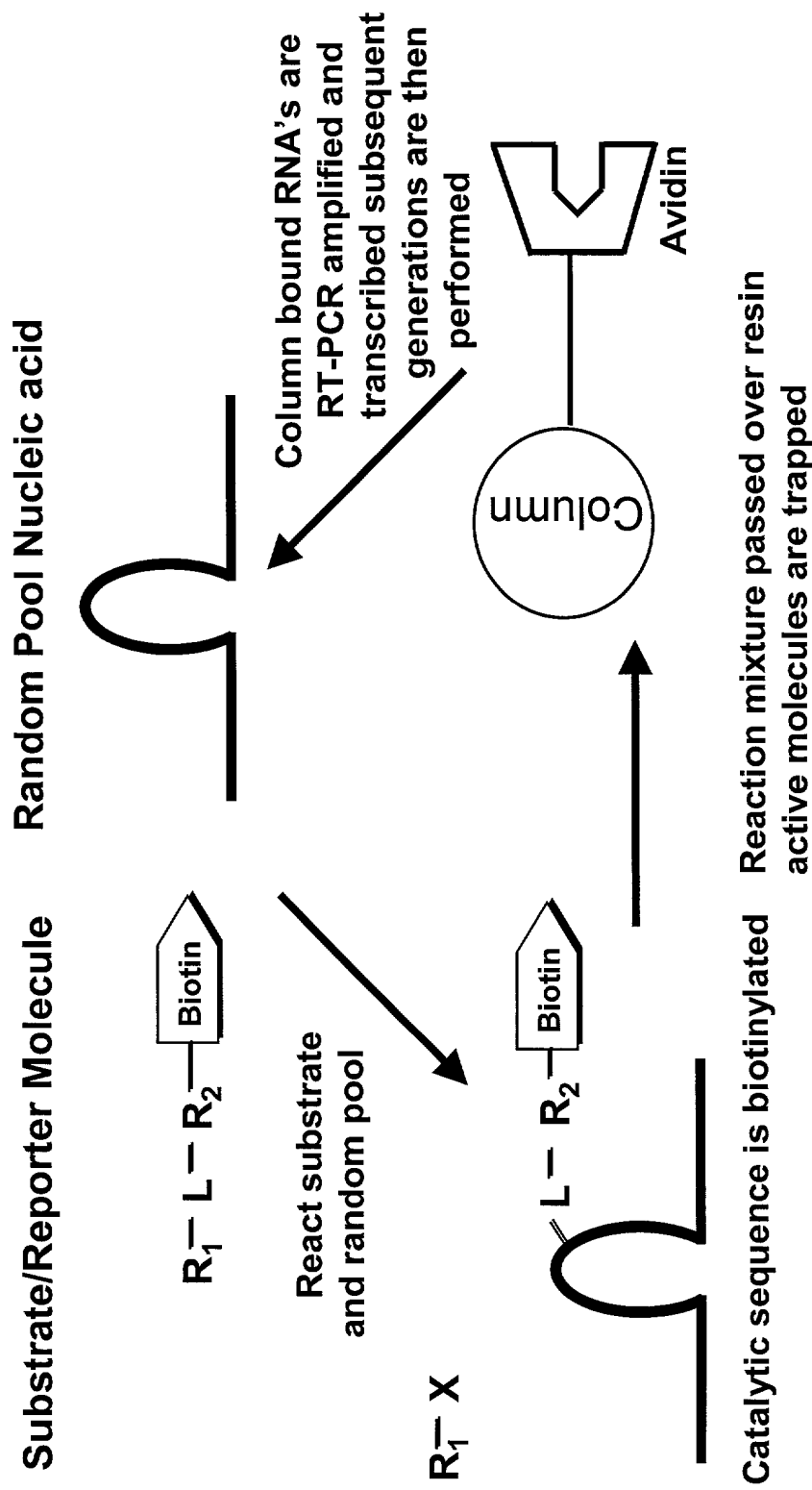


Figure 16: Diagnostic Screen

Inhibitory Folding with Target Rescue



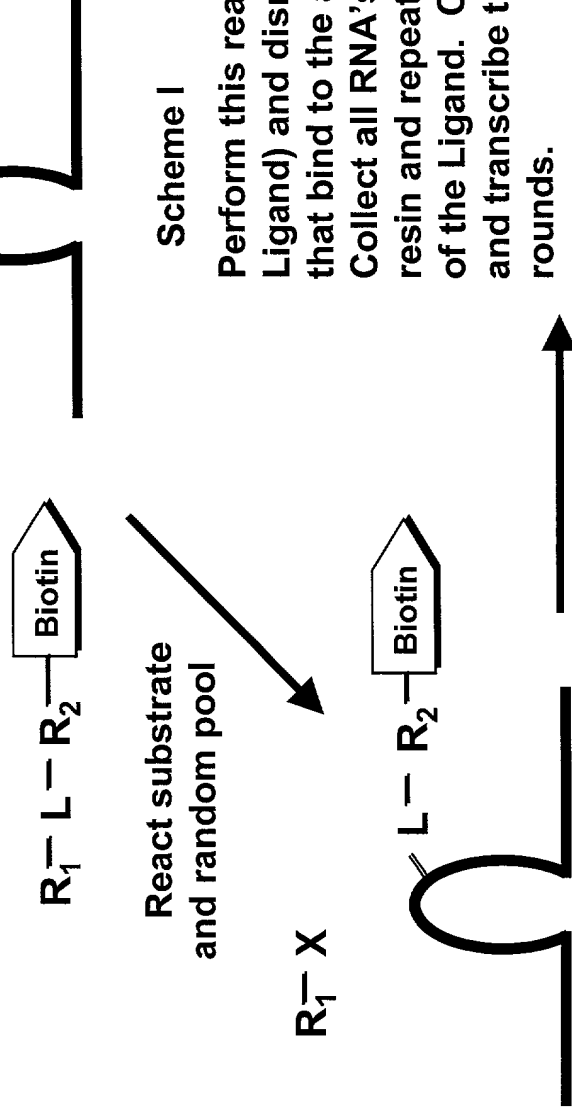
**Figure 17a: Auto-ligation Nucleic Acid Sensor Molecules - Selection Scheme**



**Figure 17b: Auto-ligation Nucleic Acid Sensor Molecules -  
Ligand Dependent**

Substrate/Reporter Molecule + Random Pool Nucleic acid

- Ligand (first round)
- + Ligand (second round)



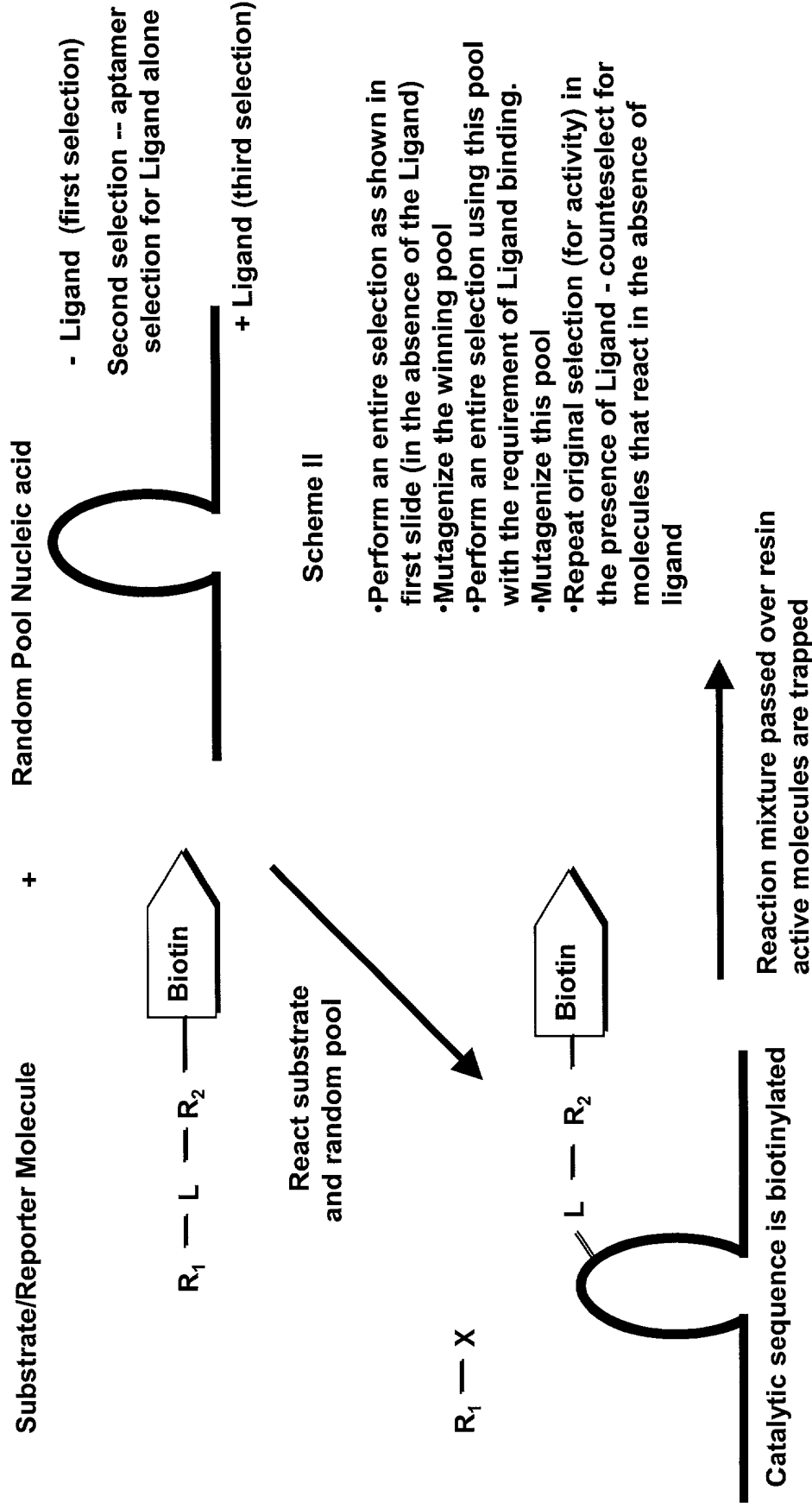
**Scheme 1**

Perform this reaction (in the absence of the Ligand) and disregard the molecules that bind to the avidin resin.  
Collect all RNA's that flow through the avidin resin and repeat the reaction in the presence of the Ligand. Collect and RT-PCR amplify and transcribe these molecules for subsequent rounds.

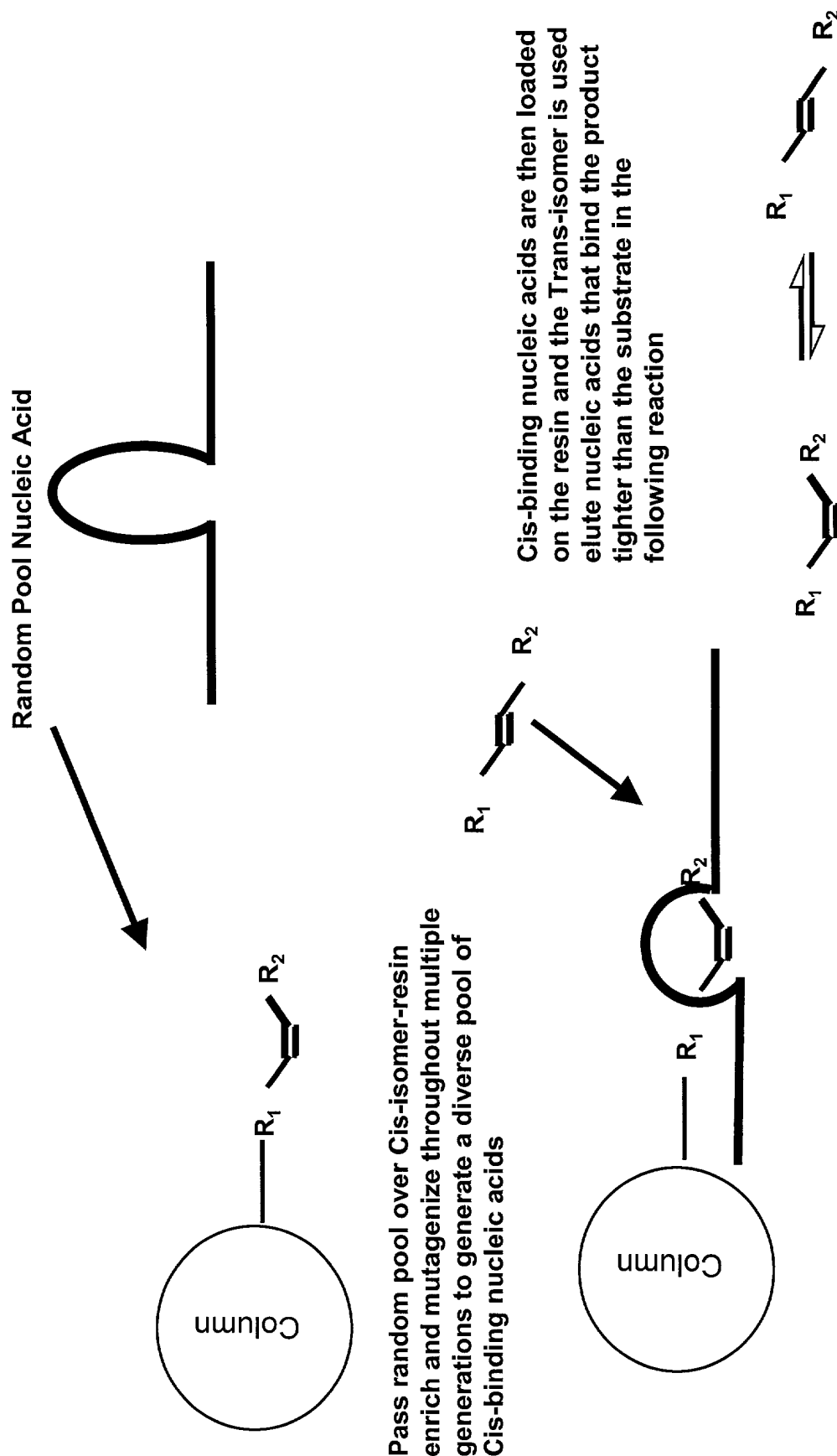
Catalytic sequence is biotinylated

Reaction mixture passed over resin  
active molecules are trapped

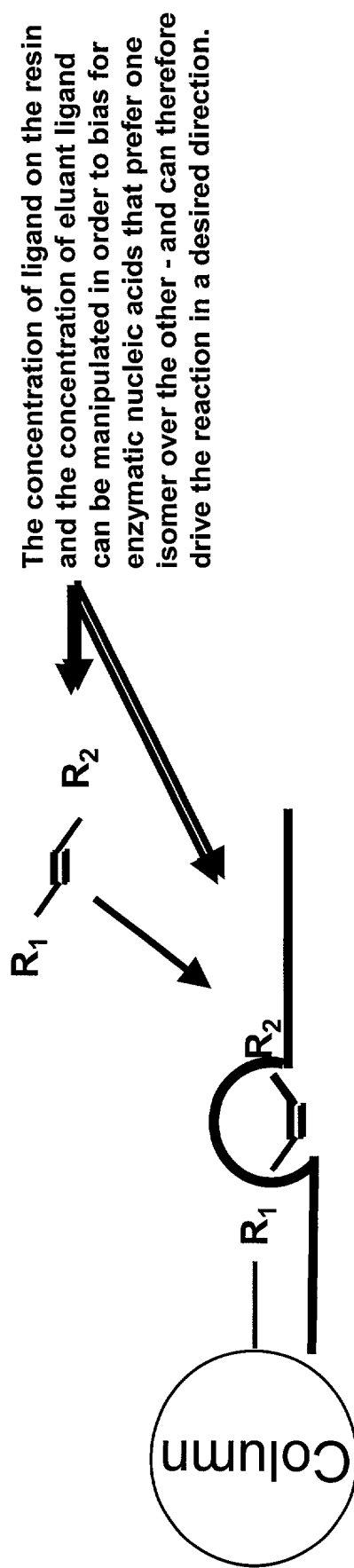
# Figure 17c: Auto-ligation Nucleic Acid Sensor Molecules - Ligand dependent



**Figure 18a: Isomerase Nucleic Acid Sensor Molecule – Selection Scheme**



## Figure 18b: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme



E.g. Selection for Cis-isomer at 100  $\mu\text{M}$  - yield  $\text{cis}K_d = 100 \mu\text{M}$   
 Elute with Trans-isomer at 0.1  $\mu\text{M}$  - yield  $\text{trans}K_d = 0.1 \mu\text{M}$

Isolate catalysts for the reaction below



**Figure 18c: Isomerase Nucleic Acid Sensor Molecule - Ligand dependent**

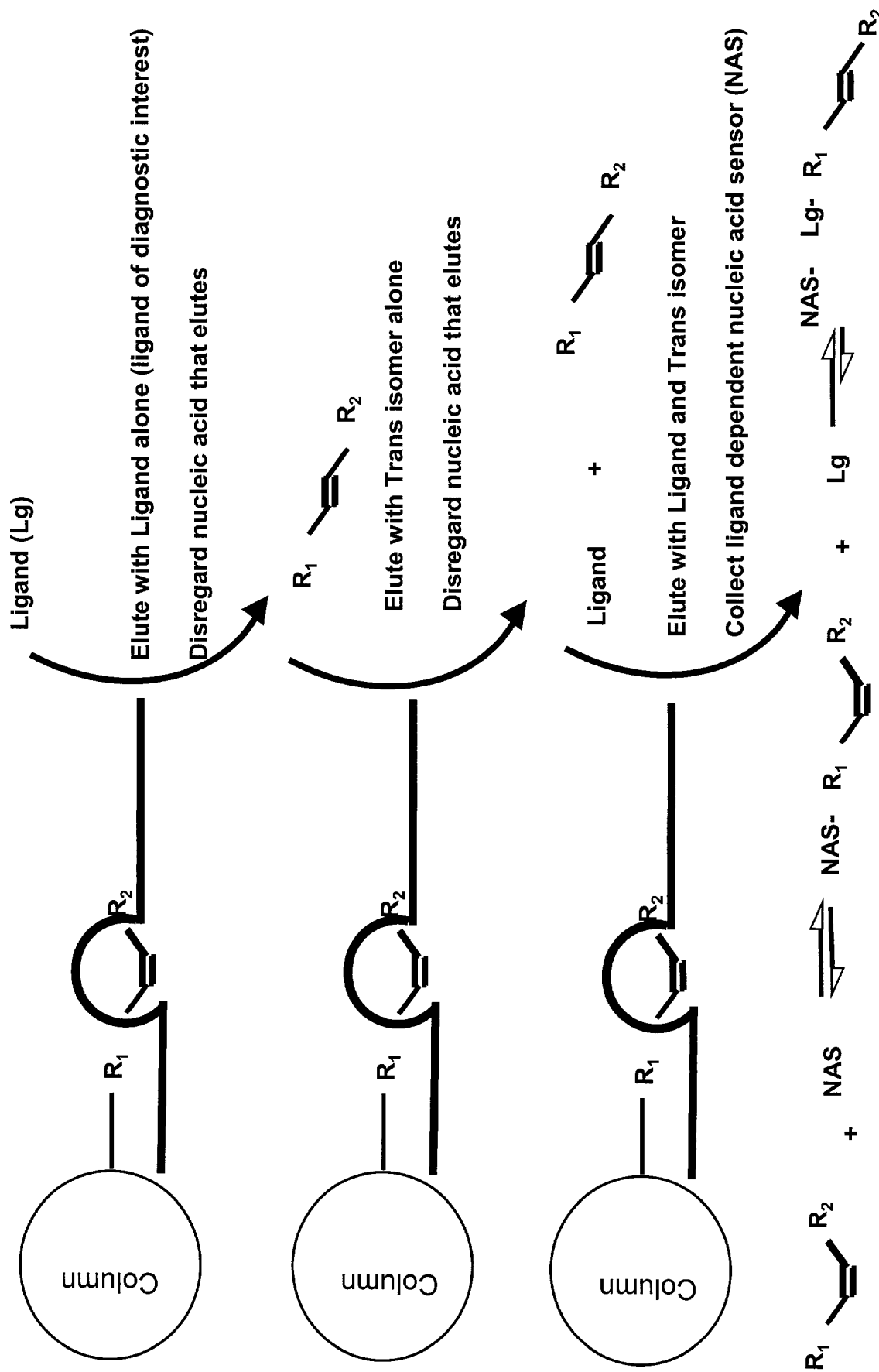
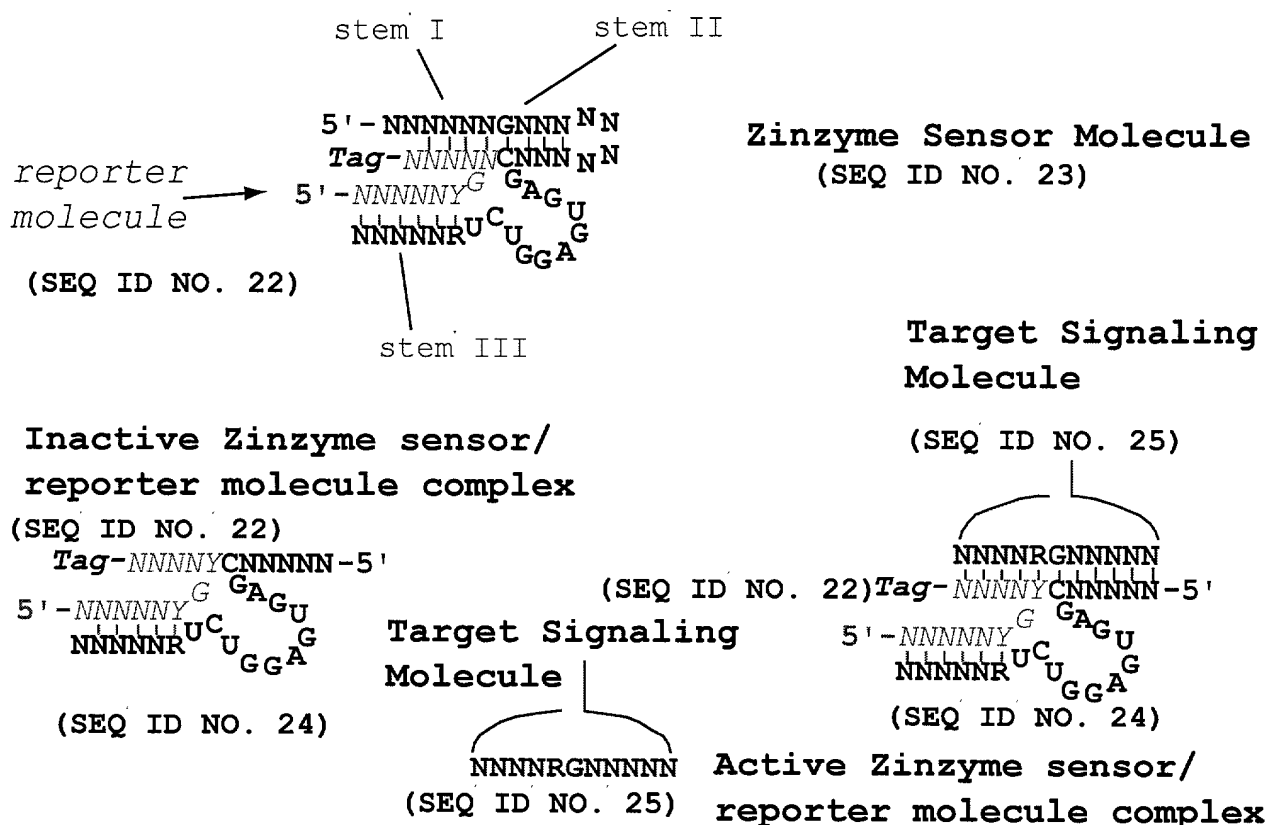
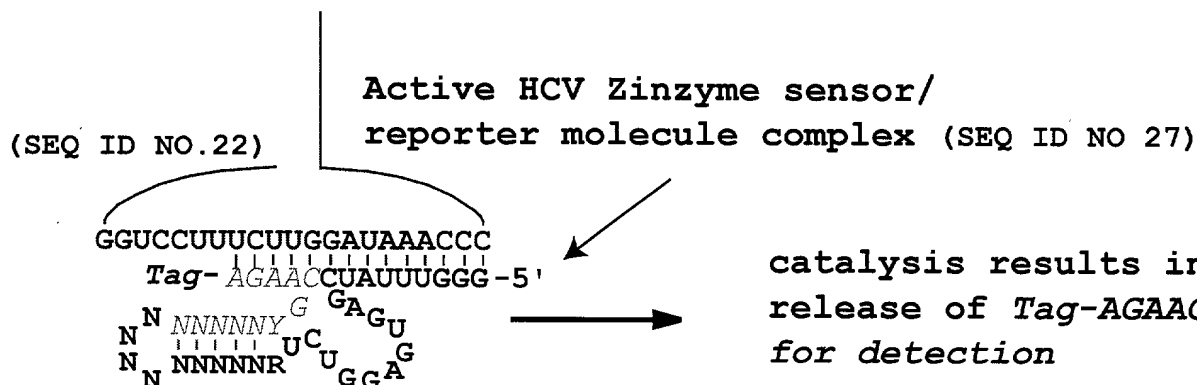




Figure 19: Zinzyme Sensor Molecule for detection of Nucleic Acid



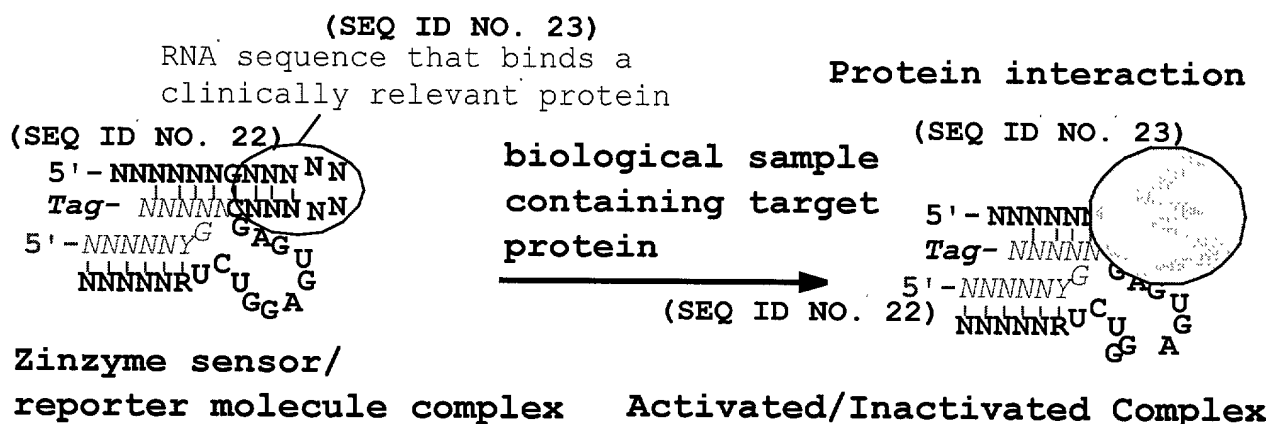
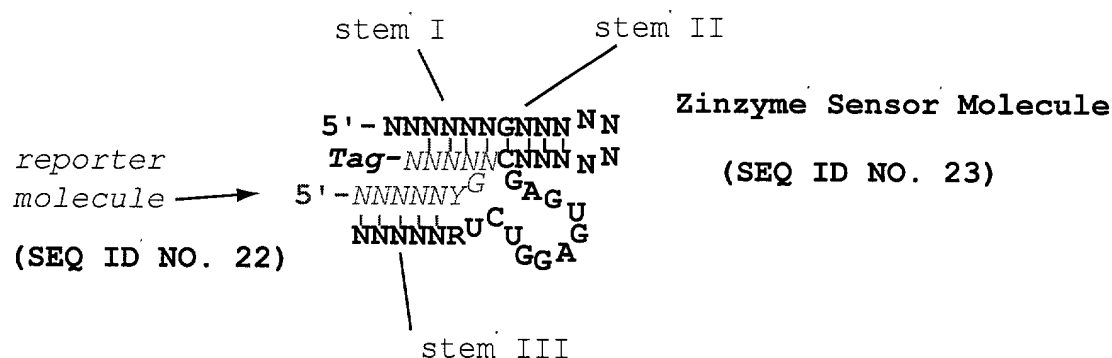
Stem-loop III of HCV (SEQ ID NO. 26)



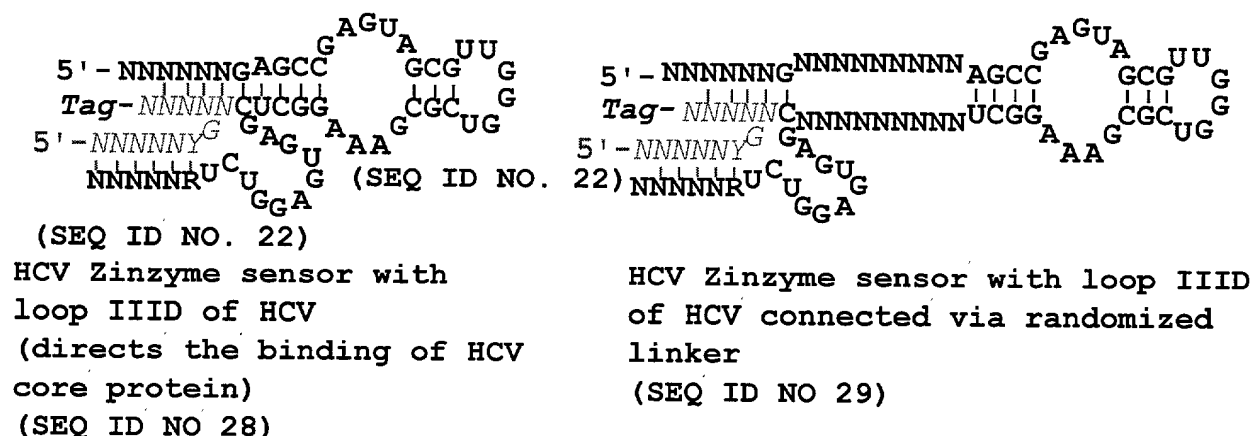
Zinzyme sensor can be attached to solid support/surface,  
 for example at the 5'-end

FIG 19: Zinzyme Sensor Molecule for detection of Nucleic Acid

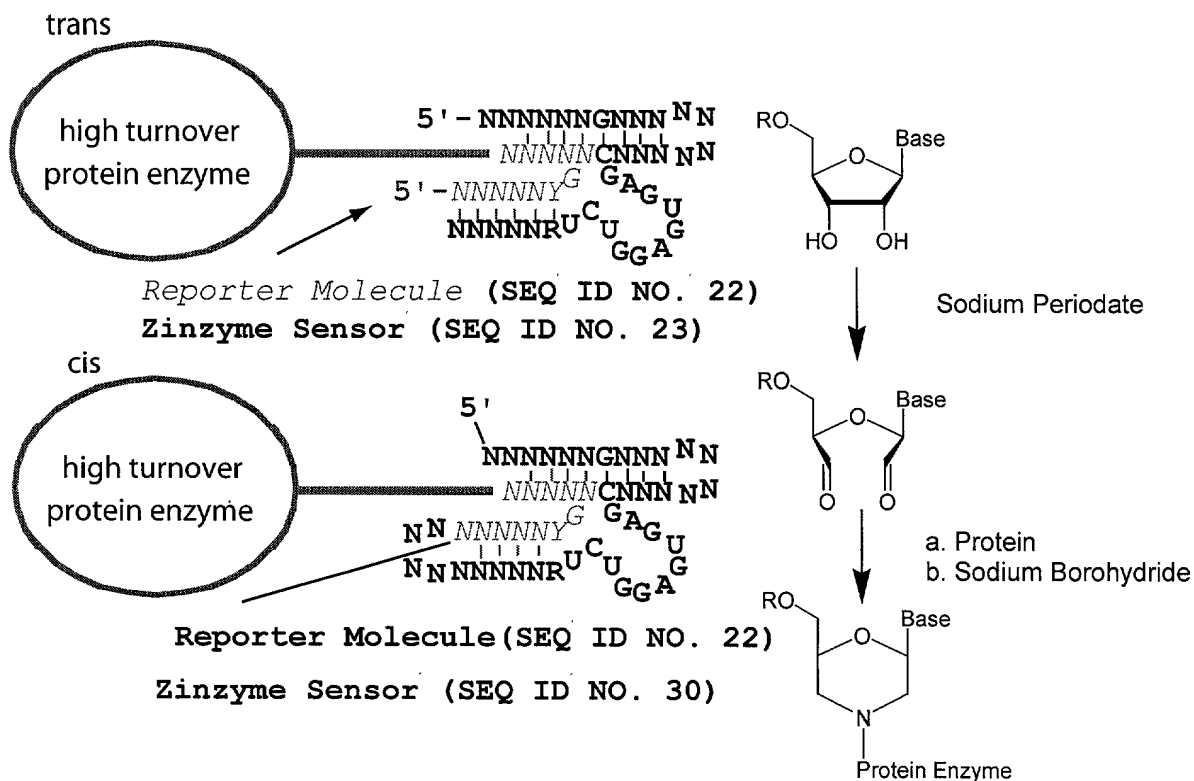
# Figure 20: Zinzyme Sensor Molecule for detection of Protein



Sensor/reporter complex for detection of HCV core protein



# Figure 21: Zinzyme Sensor Molecule with protein enzyme reporter



R is oligonucleotide.

Protein can be attached via amino linker.

Alternately, R is phosphoramidite moiety for incorporation at 5'-end of oligonucleotide.

High turnover protein enzyme is, for example, Luciferase, Horseradish peroxidase, beta-galactosidase, alkaline phosphatase.

## Amplification of signal via use of protein enzyme conjugate

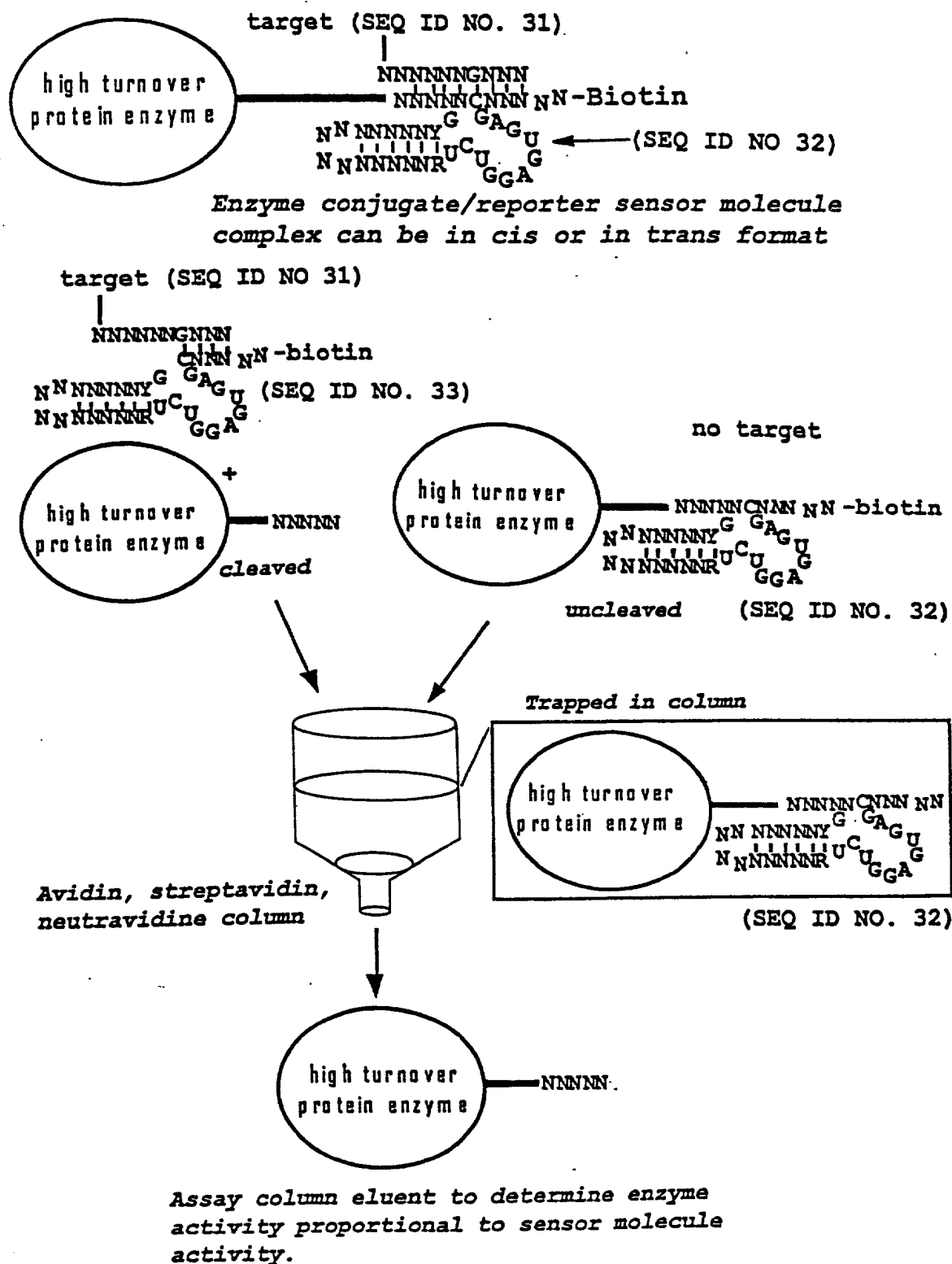
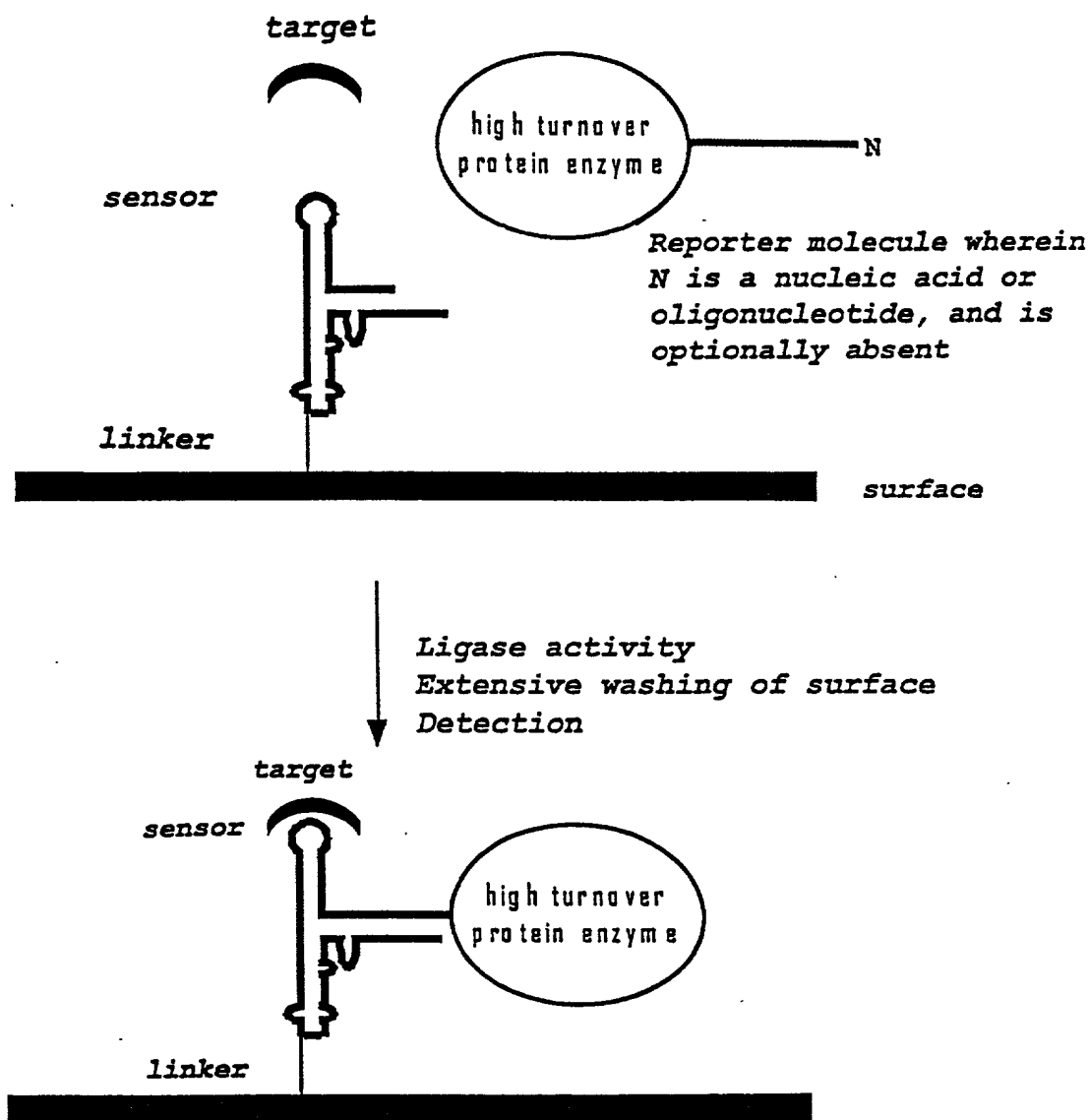


FIG. 22

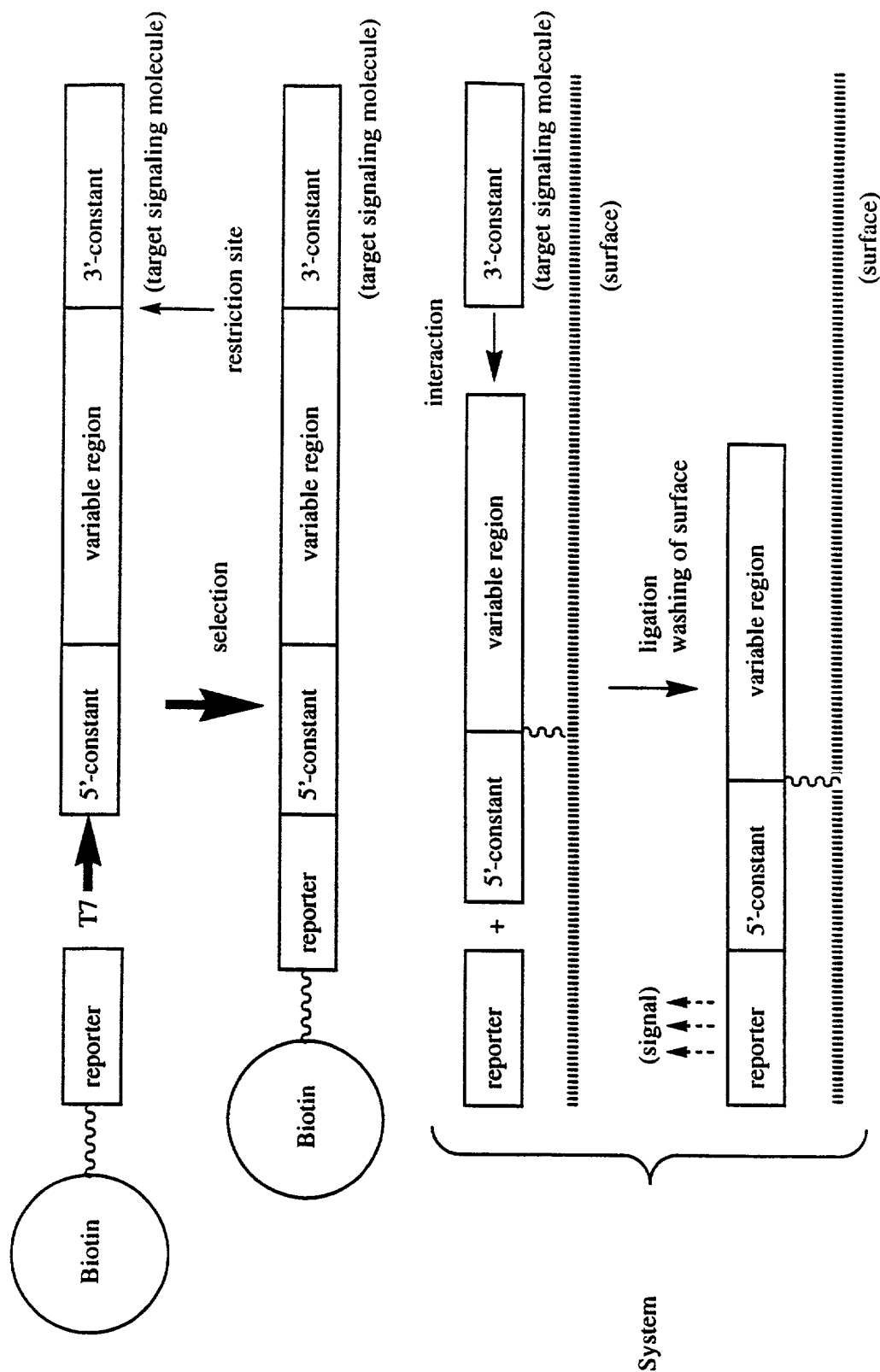
# *Ligase Sensor Molecule with enzymatic reporter*



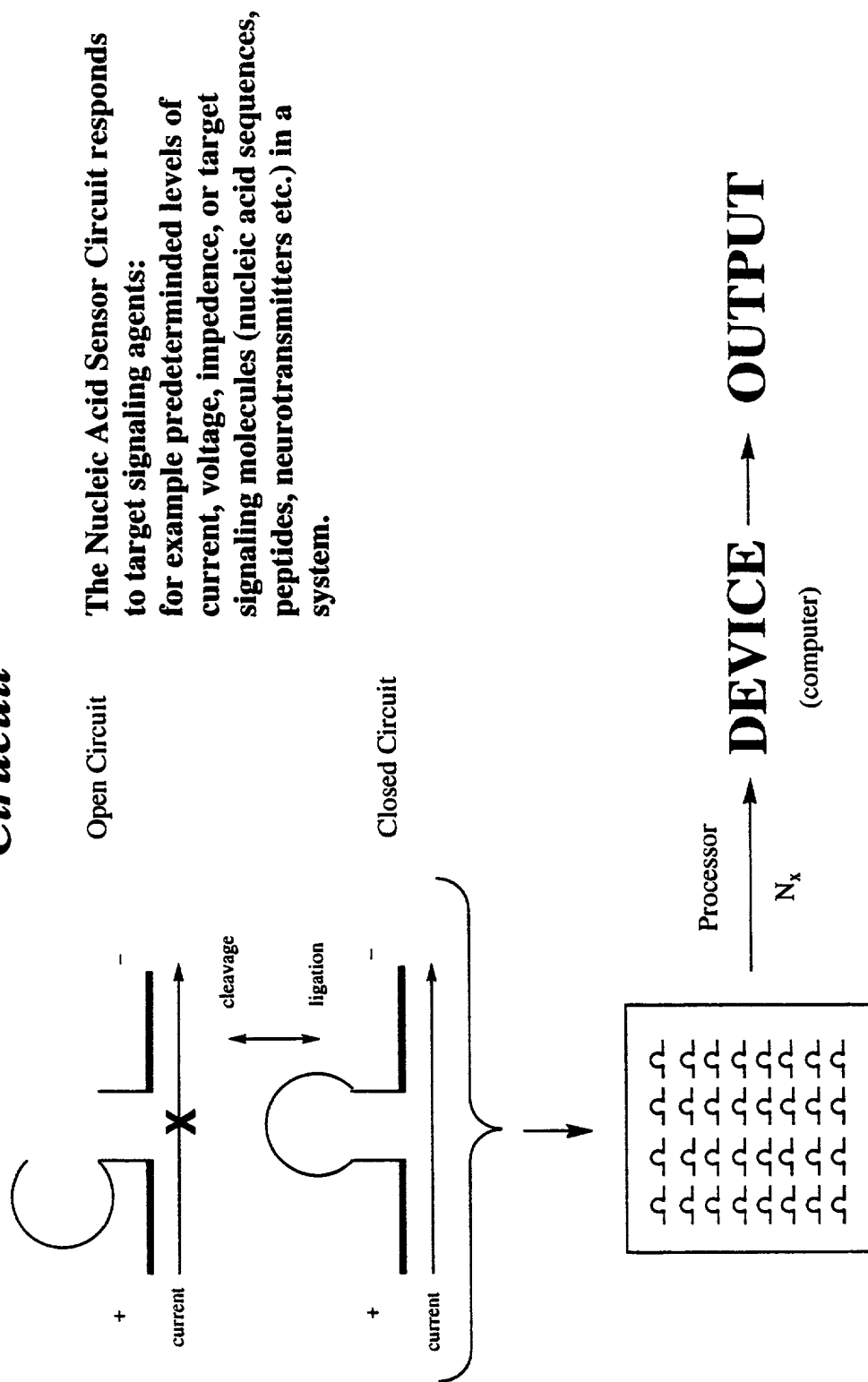
Alternatively, a fluorescent or chemiluminescent based reporter molecule is used.

FIG. 23

**Figure 24: Selection of Nucleic Acid Sensor Molecules with Ligase Activity**



**Figure 25: Nucleic Acid Sensor Molecule-Based Electric Circuit**



# ACTIVE

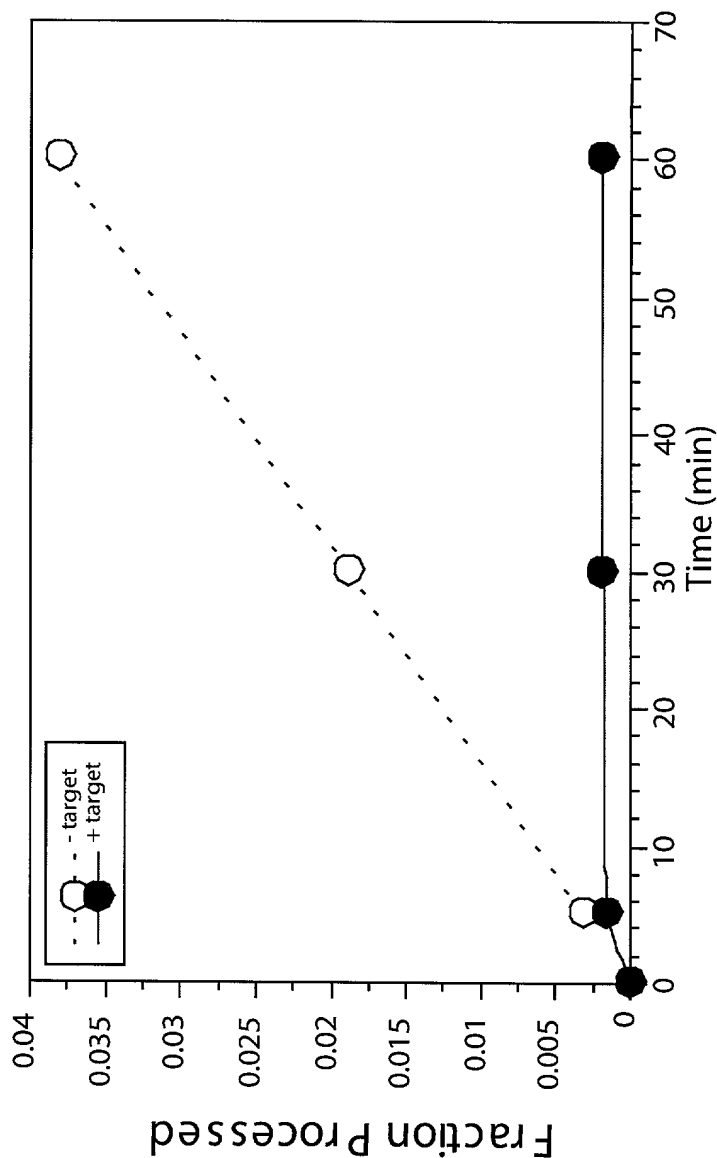




Figure 27: Target Activation of Zinzyme Sensor Molecule

10907-0372550

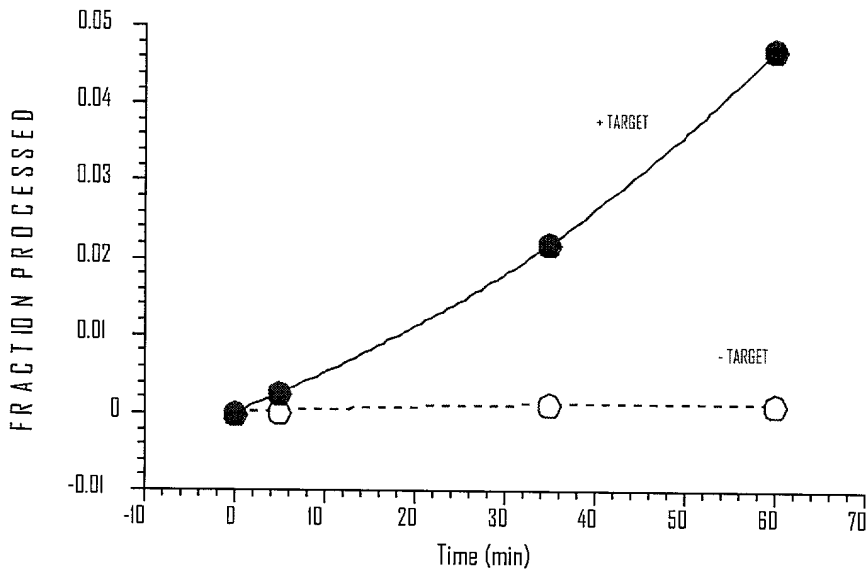
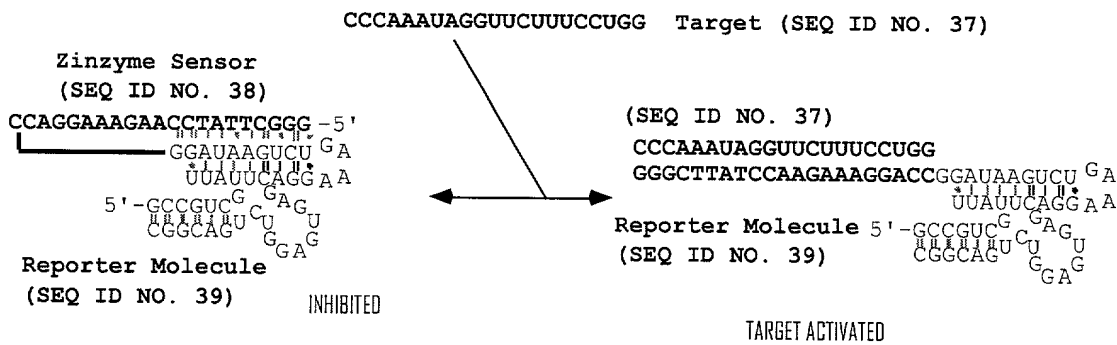
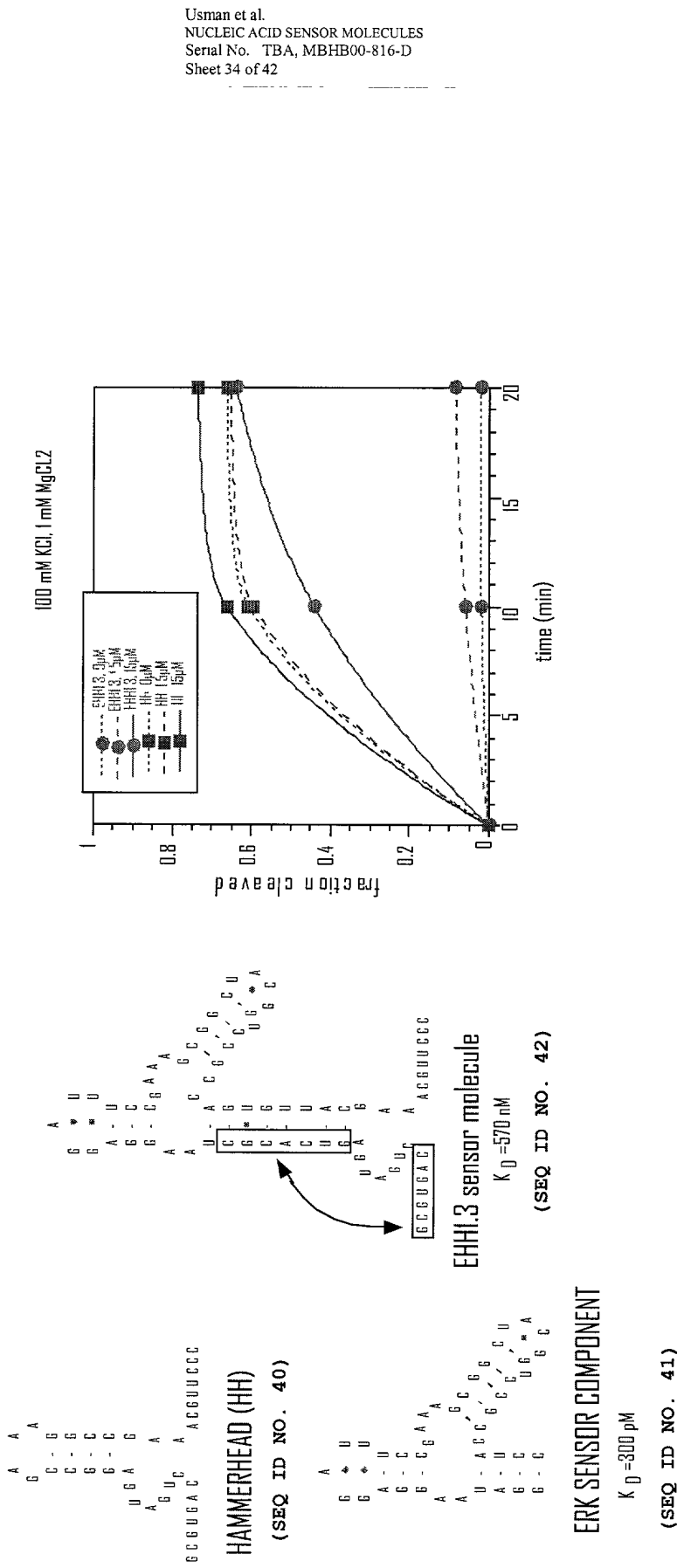


Figure 28: Erk modulated Nucleic Acid Sensor Molecule



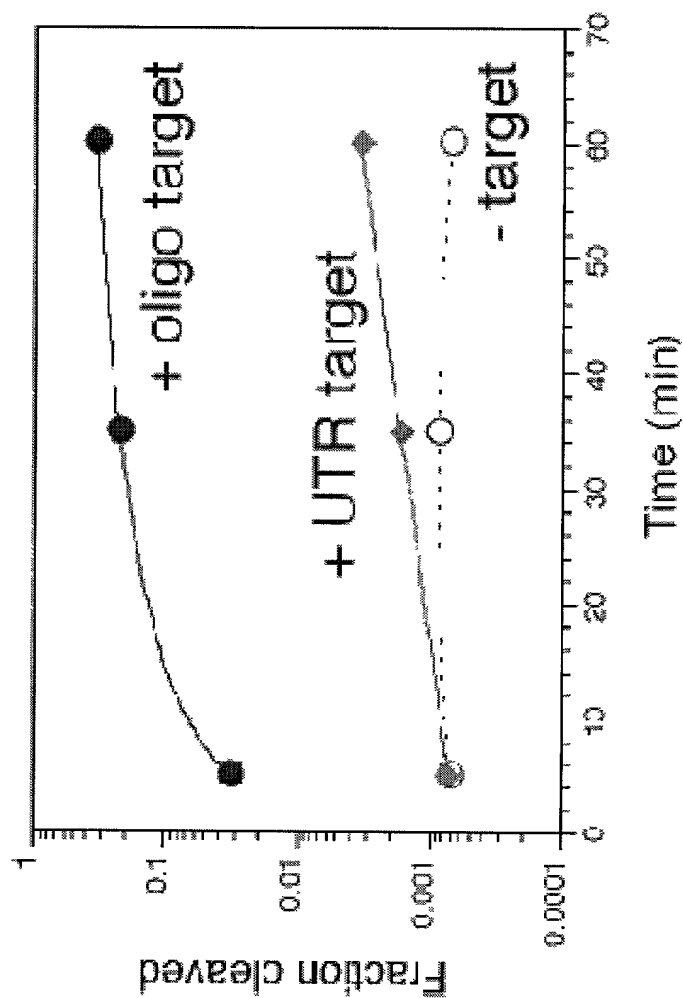
[illegible]

Figure 30

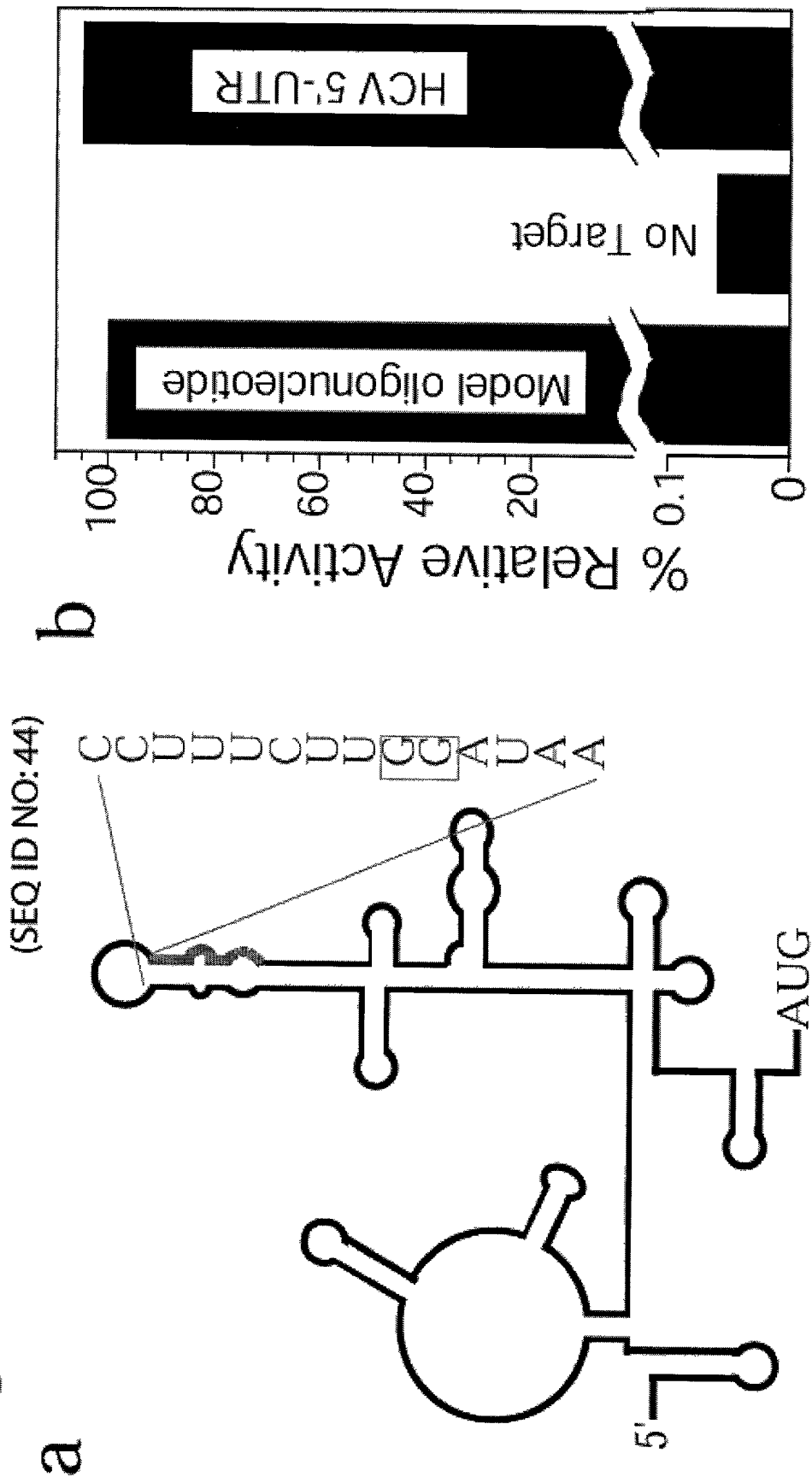


Figure 31

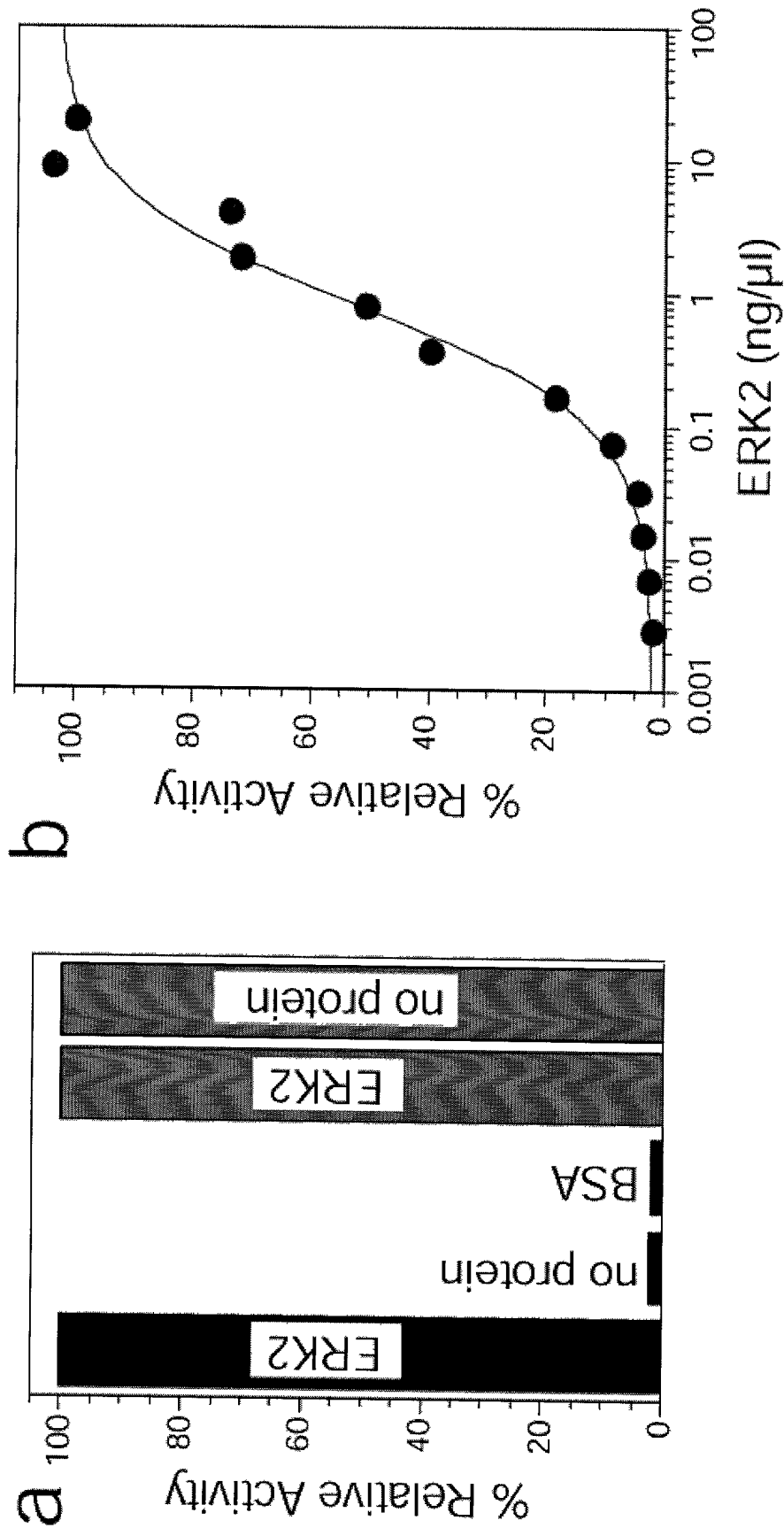
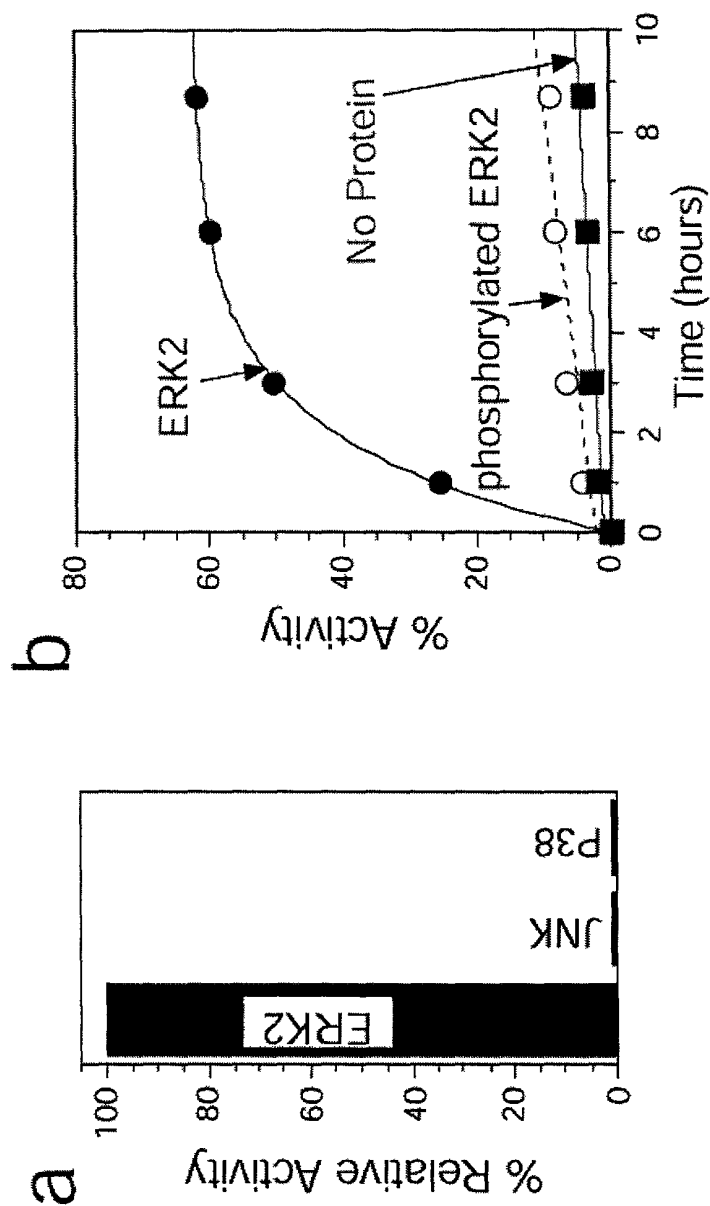


Figure 32



3' OH

A<sup>3</sup> (SEQ ID NO: 46)

(SEQ ID NO: 45) 5'

(SEQ ID NO 47)

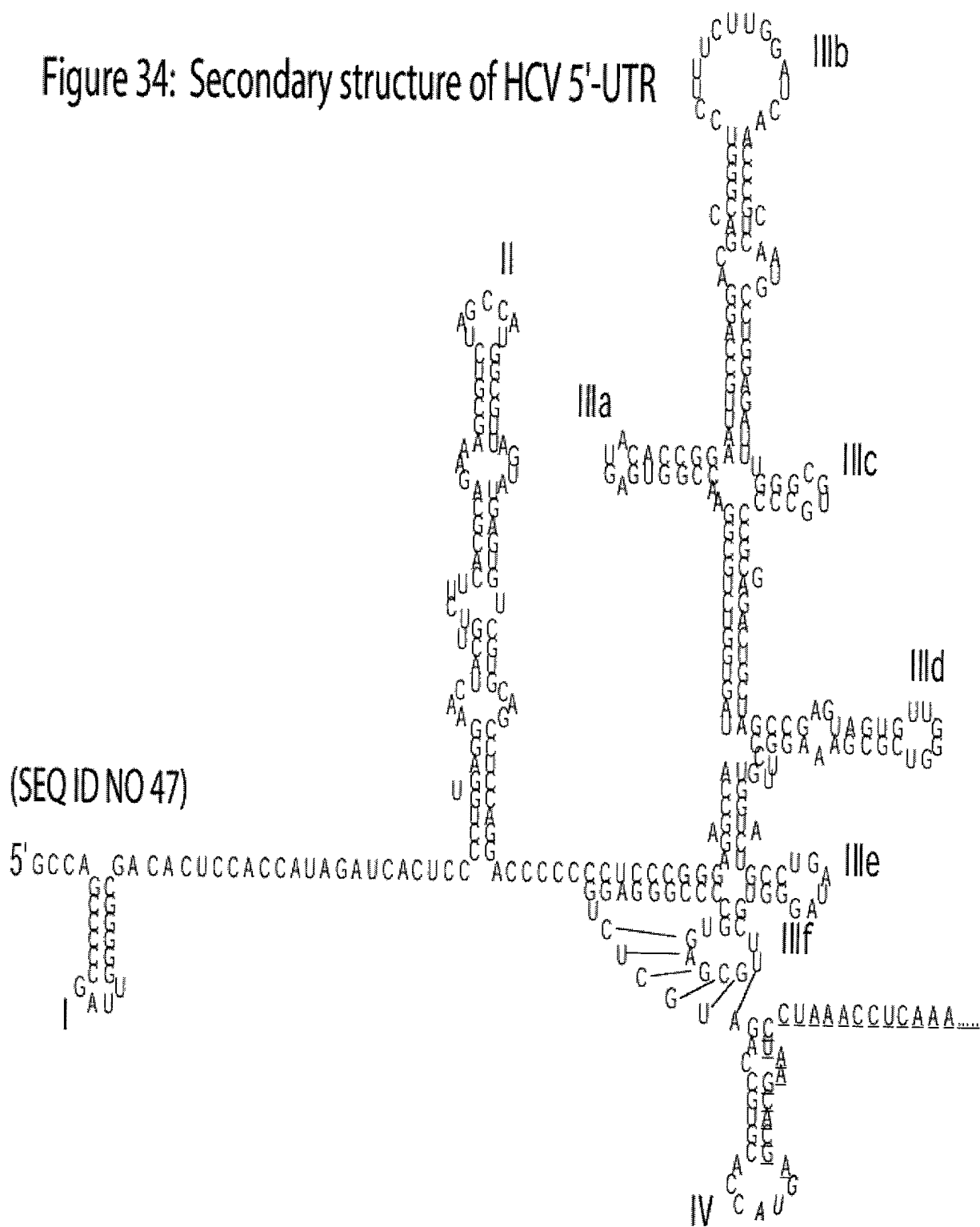




Figure 35  
Design of SNP Detection using Halfzyme-AZB7.1

|   |  | SEQ ID NO:   |  |
|---|--|--|--|
|   |  | aG G <sub>u</sub> g a <sub>g</sub> C <sub>g</sub> g a c g g g c <sub>g</sub> g <sub>g</sub> c <sub>g</sub> g a c g g g -3' |  |
| AZB7.1                                  |  | 5'-a g c g C <sub>g</sub> g a c g g g c <sub>g</sub> g <sub>g</sub> c <sub>g</sub> g a c g g g -3'                         |  |
| Target HBV 1887(=AZB7-GG 3'-T C G C G - |  | G C T G C C C C-5' (SNPT-1)  |  |
| AZB7-AG 3'-T C G C A -                  |  | G C T G C C C C-5' (SNPT-2)  |  |
| AZB7-TG 3'-T C G C T -                  |  | G C T G C C C C-5' (SNPT-3)  |  |
| AZB7-CG 3'-T C G C C -                  |  | G C T G C C C C-5' (SNPT-4)  |  |
| AZB7-GA 3'-T C G C G -                  |  | A C T G C C C C-5' (SNPT-5)  |  |
| AZB7-GT 3'-T C G C G -                  |  | T C T G C C C C-5' (SNPT-6)  |  |
| AZB7-GC 3'-T C G C G -                  |  | C C T G C C C C-5' (SNPT-7)  |  |
| RNA HBV 1433                            |  | 3'-U C G C G - G C U G C C C C-5'  |  |

**Figure 36: Single Nucleotide Polymorphism (SNP) Detection**

